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LAVOURAS TRANSGÊNICAS

Riscos e incertezas

Mais de 750 estudos desprezados pelos órgãos reguladores de OGMs

Ministério do Desenvolvimento Agrário

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Brasília, 2015

Dedicatória

à professora Magda Zanoni

Esta publicação representa a continuidade do trabalho iniciado por Magda Zanoni, no Ministério do Desenvolvimento Agrário (MDA), no sentido de discutir os riscos das plantas transgênicas para a saúde e o meio ambiente. Em sua atuação no MDA e como representante do Ministério na CTNBio, Magda dedicou esforços para chamar a atenção à importância desse debate.

Magda atuou por muitos anos como pesquisadora do Núcleo de Estudos Agrários e Desenvolvimento Rural (NEAD/MDA), onde publicou vários livros. Entre eles, em 2007 coorganizou "Plantas Geneticamente Modificadas: riscos e incertezas", que foi o embrião do presente trabalho.

Além de seu envolvimento direto com a temática das biotecnologias, Magda sempre defendeu a necessidade de um "bom uso" da ciência, com visão transdisciplinar e propondo a redistribuição justa dos seus benefícios, em especial para as populações camponesas, que desenvolveram as agriculturas de hoje como serviço perene para a humanidade.

Sua trajetória acadêmica veio sempre acompanhada de uma incansável militância social, inscrevendo e alimentando discussões sobre uma "Ciência Cidadã". Dedicou-se, por vários anos, a aprofundar o diálogo e a concretizar a cooperação entre o Brasil e a França, entre instituições acadêmicas e, também, entre organizações de agricultores. Sua atuação pautava-se por ações inovadoras e na busca pela construção de novas abordagens sobre a ciência, em particular nas articulações entre pesquisa e formação.

Graduada em Ciências Naturais e Ciências Biológicas e Geológicas pela Universidade Federal do Rio Grande do Sul (UFRGS), Magda continuou sua formação acadêmica na França, em decorrência de um exílio forçado, pela sua militância de resistência à ditadura militar e em defesa da redemocratização do Brasil. Lá, obteve diversos títulos, entre eles o de Doutorado em Sociologia do Desenvolvimento, na Universidade Paris I – Sorbonne. Aposentou-se como *Maître de Conférence* na Universidade Paris VII. Trabalhou durante muitos anos como pesquisadora do LADYS – Laboratório de Dinâmicas Sociais e Recomposição de Espaços, da Universidade de Paris *Ouest Nanterre La Defense.*

Magda nos deixou em 10 de março de 2015 e este trabalho lhe é dedicado.

Prefácio

Pelo quinto ano consecutivo, o Brasil ocupa a posição de segundo maior produtor de plantas transgênicas no mundo, perdendo apenas para os Estados Unidos. A área desse tipo de cultivo já ultrapassa os 40 milhões de hectares em nosso país.

O aumento contínuo da área plantada com lavouras de soja, milho e algodão transgênicos, e a sucessiva liberação comercial de novos organismos geneticamente modificados (OGMs), inclusive agora combinados, apontam a importância de se monitorar seus possíveis impactos sobre o meio ambiente, em geral, e os seres humanos, em particular.

Nesse sentido, o Ministério do Desenvolvimento Agrário (MDA) traz a público a presente obra, que oferece subsídios à qualificação de processos de avaliação de risco associados ao cultivo de lavouras transgênicas e ao consumo de seus produtos e derivados. A publicação, que se destina a gestores de risco, pesquisadores, profissionais das áreas biológicas, jurídicas e econômicas, bem como aos demais interessados na temática, resulta de esforço do Grupo de Estudos em Agrobiodiversidade (GEA).

O extenso trabalho de sistematização contido no livro permitiu reunir referências de artigos científicos disponibilizados em fontes variadas, disponíveis para consulta via Internet, em sites de publicações científicas ou bancos de dados. As referências apresentadas correspondem a estudos publicados por pesquisadores independentes em revistas e periódicos indexados. Grande parte dos artigos está acessível para leitura gratuita e *download*.

Nas mais de 750 indicações de textos oriundos de renomados institutos de pesquisa estabelecidos em várias regiões do planeta, cientistas alertam para os riscos e incertezas envolvidos na liberação ambiental massiva de plantas transgênicas. Evidenciando a ausência de consenso científico com relação aos impactos dos transgênicos sobre a saúde das pessoas e para a socioecobiodiversidade, o livro traz elementos para o exame de problemas decorrentes do uso desse tipo de biotecnologia. O acúmulo de informações aqui recolhidas sobre suas implicações ambientais, econômicas, sociais e morais, reforçam a necessidade de análises críticas do atual modelo de desenvolvimento, abrindo a discussão para a busca de caminhos alternativos para o meio rural.

Com o livro “Lavouras Transgênicas – riscos e incertezas: mais de 750 estudos desprezados pelos órgãos regulardores de OGMs”, o intuito do MDA é, portanto, promover a reflexão e contribuir ao debate sobre a liberação e uso de plantas transgênicas, tendo como foco a importância da manutenção da biodiversidade e da agrobiodiversidade, da segurança e da soberania alimentar e do desenvolvimento rural sustentável.

Boa leitura!

Patrus Ananias
Ministro de Estado do Desenvolvimento Agrário

Apresentação

Este livro tem um formato diferente dos livros convencionais que se propõem a apresentar uma revisão bibliográfica de publicações científicas relativas a determinado tema. Inova ao colocar questionamentos sobre aspectos do debate científico no campo da transgenia para, em seguida, apresentar um elenco de referências bibliográficas que contrariam versões desse debate adotadas por agências reguladoras e divulgadas em campanhas de marketing das empresas produtoras de transgênicos.

Todas as referências aqui expostas correspondem a estudos publicados por pesquisadores independentes em revistas e periódicos indexados. Esses artigos permitem problematizar aqueles temas examinados, levando em conta argumentos por vezes radicalmente opostos aos produzidos pelas empresas dos setores agroquímico e biotecnológico ou a seu serviço.

Pesquisadores, estudantes, formadores de opinião e aqueles alinhados aos grupos que acreditam ou tem interesse em defender a hipótese da ausência de riscos para a saúde e o meio ambiente no uso comercial das plantas transgênicas, terão aqui oportunidade de rever suas opiniões. Aos demais, este documento oferece elementos de suporte para abalizar o debate com os primeiros, ao se tratar de defender os interesses da sociedade, da preservação da natureza e de um modelo agrícola sustentável.

A revisão bibliográfica aqui empreendida abrange riscos para a saúde humana e animal, riscos ao meio ambiente, bem como problemas agronômicos ou mesmo socioeconômicos. Entre os exemplos incluem-se evidências de recrudescimento de ataques de insetos resistentes às toxinas Cry[1] e multiplicação de populações de plantas ruderais[2] tolerantes aos herbicidas. Também são discutidos impactos

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[1] Proteínas com propriedades inseticidas que resultam na morte de determinados insetos quando consumidas.

[2] Também erradamente chamadas de plantas “daninhas”.

de toxinas e de agrotóxicos associados às plantas geneticamente modificadas.

O livro examina questões de biossegurança focalizando aspectos que têm sido negligenciados pela maioria das agências de avaliação do risco de transgênicos, tais como a Comissão Técnica Nacional de Biossegurança (CTNBio) e seus órgãos de registro e fiscalização, a Anvisa e o Ibama, no caso específico do Brasil. Os elementos aqui expostos em cerca de 750 estudos validados por revistas científicas com conselho editorial mostram claramente que não há consenso na comunidade científica sobre o tema da transgenia e seus impactos.

Tal falta de consenso, objeto dos muitos alertas do Grupo de Estudos em Agrobiodiversidade (GEA), ligado ao Ministério do Desenvolvimento Agrário (MDA), em suas diversas publicações, seminários e debates ao longo dos últimos dez anos, vê-se agora reforçado pela farta documentação bibliográfica aqui reunida.

A iniciativa desse livro, por seu ineditismo, cumpre portanto papel de interesse público ao chamar atenção para as responsabilidades difusas na sociedade, porém concentradas na CTNBio e no Conselho Nacional de Biossegurança (CNBS), a quem caberia revisar o embasamento científico adotado por aquela Comissão, cujos pareceres tem sido reiteradamente favoráveis aos pleitos das empresas desenvolvedoras de transgênicos. Assumindo um consenso que inexiste no meio científico, esses pareceres ampliam a magnitude dos riscos a que se submetem a população e os biomas brasileiros, desconsiderando-se o Princípio da Precaução e comprometendo a credibilidade do governo e suas instituições. Assim, infelizmente, não é apenas a Ciência que é prejudicada pelo obscurantismo científico.

Grupo de Estudos em Agrobiodiversidade (GEA/NEAD/MDA)

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Introdução

O livro "Lavouras Transgênicas – Riscos para a Saúde e Meio Ambiente – mais de 750 estudos desprezados pelos órgãos reguladores de OGMs" incorpora resultados de vasta pesquisa bibliográfica cobrindo o tema dos riscos e das incertezas associados ao uso de plantas transgênicas.

Foram examinadas as principais publicações científicas referendadas por corpos editoriais especializados, atinentes ao cultivo de plantas transgênicas (em escala comercial ou experimental[3]), ao manejo daquelas lavouras e ao consumo animal e humano de seus produtos.

Destacadas as questões relativas à biossegurança, privilegiando aspectos de saúde ambiental, humana e animal associados ao uso das tecnologias em questão, contabilizamos cerca de 750 artigos referenciados, publicados entre os anos 1980 e 2015. Mantidos na invisibilidade porque seus achados contrariam as abundantes campanhas de marketing pró-ogms, estes estudos lançam novas luzes sobre o tema.

A abundância e a importância desses documentos, bem como a invisibilidade que lhes tem sido imposta nas discussões levadas a termo pelas agências reguladoras, justificam esta publicação, cujo objetivo é alimentar a polêmica científica quanto à biossegurança das plantas transgênicas. Trata-se, como os documentos aqui reunidos evidenciam, de debate forte e ativo, em que pesem as tentativas de imposição de falsos consensos por parte da indústria de biotecnologia e dos seus lobbies associados. Ao contrário do apregoado por fundações, institutos, associações e ONGs que incorporaram o ideário das empresas de biotecnologia mediante apoios orçamentários e membros em seus conselhos de administração[4], a comunidade científica permanece profundamente dividida.

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[3] Em geral denominados de liberações planejadas no meio ambiente (LPMA).

[4] No caso do Brasil podemos citar o Conselho de Informação em Biotecnologia (CIBio) e, em nível internacional, o Internacional *Life Science Institute* (ILSI).

A polêmica, que abrange a totalidade dos temas relativos à biossegurança das plantas transgênicas, pode ser resumida em termos de riscos para a saúde humana e animal e riscos para o meio ambiente, subdividindo-se em subtemas e particularidades associadas aos transgenes envolvidos, aos organismos hospedeiros, aos pacotes tecnológicos e agrotóxicos associados, entre outros. Como será demonstrado ao longo deste livro, centenas de estudos evidenciam riscos e fragilidades de afirmativas que alegam ausência de toxicidade das proteínas Bt para seres humanos e animais de criação. O mesmo se verifica quanto ao impacto de subdosagens e os reflexos de efeitos subletais das toxinas Cry sobre organismos não alvo ou quanto aos danos provocados pelas lavouras tolerantes a herbicidas sobre a microbiota do solo e os agroecossistemas em que se inserem.

Também são questionáveis as afirmações sobre a impossibilidade de contenção do fluxo gênico entre espécies GM e destas com espécies nativas ou agrícolas. Os impactos sobre a agrobiodiversidade são tanto maiores quanto mais vastas as possibilidades, os mecanismos e os vetores de polinização, mas não se restringem a isto. Os estudos também revelam possibilidades de transferências horizontais de genes e seus fragmentos, ampliando os riscos decorrentes da superação de barreiras naturais entre as espécies.

Merecem destaque, ainda, a expansão no uso de pesticidas associados às lavouras transgênicas, à periculosidade dos herbicidas à base de glifosato, do glufosinato de amônio e de outros agrotóxicos que sucedem aos primeiros em espiral de toxicidade crescente, bem como os processos de avaliação do risco praticados pelos órgãos reguladores.

Chamamos atenção para o fato de que seria necessário um trabalho de pesquisa similar – e não menos amplo – para abordar o conjunto de riscos socioeconômicos que tais plantas representam. Análises efetivas de performances agronômicas, com balanço de custos e benefícios levando em conta perspectivas de médio prazo, jamais foram realizadas de forma isenta e independente, levando em consideração a possibilidade de rejeitar essas tecnologias. O objetivo de estabelecer um balanço sistêmico, cotejando risco/benefício da biotecnologia aplicada ao domínio da agricultura, o que deveria balizar as decisões – no Brasil – do Conselho Nacional de Biossegurança, jamais esteve entre as preocupações da CTNBio. Se perseguido, esse objetivo com certeza deveria apoiar-se não apenas nos estudos aqui reunidos mas também agregar a esses aqueles gerados em outro universo de revistas científicas especializadas, de forma a cobrir os temas da economia e da sociologia.

A forma de apresentação deste livro também difere dos formatos usuais de livros nos quais se indicam nos textos as citações bibliográficas utilizadas. No presente caso, é feita uma explicação do tema abordado e elencadas as citações com referências para que o leitor possa acessar na íntegra as publicações originais e usá-las como bem queira e chegar a conclusões próprias sobre os diversos temas abordados.

Esperamos que este esforço cumpra a finalidade de desafiar estudiosos daqueles campos a complementar esta pesquisa, de maneira a consolidar o leque de conhecimentos necessários ao Conselho Nacional de Biossegurança[5] (CNBS) para bem cumprir seu dever de avaliar a oportunidade e a conveniência de apoiar as decisões supostamente científicas da CTNBio, que, ignorando este acervo, invariavelmente recomenda autorização irrestrita para o plantio e o consumo (humano e animal) de transgênicos no Brasil.

A primeira parte desta publicação, intitulada **Efeitos imprevisíveis e não intencionais da transgenia**, focaliza estudos que apontam para a ausência de controle do biotecnólogo sobre as ações e os mecanismos que utiliza, ao impor ao organismo transformado uma nova função biológica. Apesar de aleatória em termos de processo constru-

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[5] Instância política de nível superior às decisões da CTNBio, legalmente competente para análise do risco socioeconômico das plantas transgênicas bem como para avaliar a oportunidade e a conveniência da tecnologia. Composto por onze ministros, se reuniu apenas duas vezes após recursos emitidos pelo Ibama e pela Anvisa contra decisão técnica da CTNBio a respeito dos primeiros milhos transgênicos liberados comercialmente, em 2008-2009. Naquelas oportunidades, reforçou o papel último da CTNBio e abriu mão das suas outras competências, sendo silencioso desde esse momento.

tivo, essa função e outras por ela comprometidas serão fixadas de maneira perene e estável em seu protagonismo sobre as gerações futuras, daquele ser e de outros por ele afetados.

As dificuldades envolvidas no ato de inserir voluntariamente (e corretamente) determinadas sequências gênicas em outros organismos por meio das técnicas de transgenia clássicas aparecem logo no início do processo de transformação genética. Estas se concretizam, entre outros exemplos, pela inserção de inúmeras cópias do transgene de interesse no organismo transformado, que se fixam, por inteiro ou por partes, em locais aleatórios do genoma do novo ser transgênico.

De fato, sem efetuar testes e estudos demorados e onerosos que permitam caracterizar a transformação obtida, os pesquisadores não saberão, a rigor, o que realizaram. O fato básico é que os mecanismos de inserção não permitem antecipar o local onde serão incorporadas as sequências genômicas desejadas, nem como isso será concluído, ou mesmo quantas delas (e em que estado, se no todo ou por partes) serão incorporadas ao DNA receptor, ou ainda qual será a reação do genoma transformado diante dessa invasão transgênica. Também não há como antecipar se a planta aceitará os transgenes e suas funções, reparando como possível os danos provocados pelo método no DNA, ou se, ao contrário, tratará de silenciar o transgene incorporado, bloqueando sua expressão.

Em paralelo, os pesquisadores também afirmam não haver mecanismos que permitam acompanhar a totalidade das funções genéticas e/ou metabólicas das sequências genômicas a serem inseridas. Aliás, é possível que ainda não exista arcabouço científico que permita sequer estimar, quanto mais conhecer, a totalidade das relações metabólicas envolvidas nas relações desencadeadas por um único gene, levando em conta as oscilações ambientais e o horizonte de vida de qualquer indivíduo.

O dogma central em que se apoia a transgenia, reiterado com frequência nas agências reguladoras e expandido com o pressuposto da equivalência substancial, há mais de 20 anos já se revelou falho e sem sustentação científica. Um gene não codifica uma única proteína que cumprirá uma função clara e definida. Um gene, sob influência do ambiente, tornará possível a expressão de um amplo conjunto de proteínas, que por sua vez interagirão de diferentes maneiras com o macro conjunto de proteínas do organismo transformado e suas relações com o meio.

Enfim, a própria técnica da transgenia atua de forma alheia aos conjuntos de relações envolvidas, abstraindo ou ignorando os fenômenos epigenéticos que regulam inúmeros mecanismos biológicos dos organismos, inclusive os hereditários. Ao se concentrar em menos de um único suporte da informação biológica, um fragmento do DNA, assume que os organismos resultam da simples adição de suas partes, como se, conhecendo o genoma, dispuséssemos de um mapa capaz de organizar a criação da vida. Em outras palavras, o sucesso da transgenia em transpor uma nova função a um organismo que naturalmente não a possuía, repousa antes de tudo sobre o acaso, onde a probabilidade de sucesso se revela substancialmente inferior à das loterias.

Numa segunda parte, intitulada **Questões agronômicas relativas ao cultivo de plantas transgênicas**, os artigos selecionados focalizam sobre estudos de biossegurança que observam problemas agronômicos ligados ao plantio e ao manejo de plantas transgênicas liberadas comercialmente.

Esses problemas dizem respeito ao desenvolvimento de populações de insetos e de plantas ruderais geneticamente resistentes à tecnologia transgênica -sensibilidade a proteínas Cry e a herbicidas, respectivamente, a interações biológicas existentes, potencialmente causadoras da ineficácia da tecnologia de resistência a vírus, bem como à ocorrência de distúrbios ecológicos nos agrossistemas. Todos esses aspectos resultam em diversos problemas agronômicos como ataques de pragas secundárias e disseminação transgênica em organismos geneticamente aparentados, com impactos econômicos, sociais e ecológicos que extrapolam de longe as áreas cultivadas por aqueles agricultores que optam por adotar tais tecnologias.

Cabe destacar que alguns artigos relativos à produtividade, ao uso de agrotóxicos e ao lucro econômico dos produtores de plantas transgênicas foram incluídos, apesar de fugirem levemente do escopo desta publicação. Do mesmo modo, o tema da coexistência é abordado principalmente com referência a alguns artigos que tratam do fluxo gênico, horizontal e vertical, adicionando casos de contaminação entre cultivos comerciais transgênicos e não transgênicos. Cabe salientar que o tema da "coexistência", além da consideração de estudos e argumentos biológicos que se encontram na literatura científica, necessita uma abordagem que agregue elementos sociais, econômicos e culturais próprios a cada região administrativa (país, região, etc.) e seus diferentes agroecossistemas e biomas.

A terceira parte, intitulada **Riscos para o meio ambiente associados ao cultivo e/ou uso de plantas transgênicas**, abrange um conjunto de riscos e incertezas associados ao plantio e à disseminação de plantas geneticamente modificadas no meio ambiente.

A sistematização desses artigos se estrutura primeiramente em relação aos riscos envolvendo plantas Bt. Incluem-se nesse ponto a polêmica sobre a teoria da especificidade das proteínas Bt, sua persistência no meio ambiente e nas cadeias tróficas e os impactos negativos dessas proteínas – diretos e indiretos –sobre macro-organismos não alvo (ONAs) e as comunidades da microbiota do solo.

Num segundo momento, foram sistematizados estudos que tratam das plantas tolerantes a herbicidas (TH), em especial aqueles à base de glifosato. Incluem-se aí artigos relativos aos impactos negativos dos herbicidas associados às plantas TH sobre o meio ambiente. De fato, a avaliação do risco dos herbicidas associados ao uso de plantas transgênicas oferece importantes informações de biossegurança sobre o tema dos riscos ambientais decorrentes das lavouras TH, mesmo quando o metabolismo dos venenos ocorre de forma diferente para os dois tipos de plantas (natural e GM).

Enfim, referências sobre riscos de disseminação/contaminação transgênica em espécies não agrícolas vêm complementar as questões relativas à disseminação de transgene nos agrossistemas, analisadas na segunda parte dessa publicação.

A quarta parte desse trabalho se intitula **Riscos para a saúde associados ao cultivo e/ou uso de plantas transgênicas**. Aqui estão reunidas cerca de 200 referências sobre esse tema – desde artigos que apontam insuficiência de dados científicos para concluir pela ausência de riscos à saúde, quando consumidas plantas geneticamente modificadas ou suas partes, até detalhamento sobre problemas relacionados às plantas Bt e TH, tratadas separadamente, de modo similar ao realizado nas partes dois e três deste livro.

No que diz respeito às plantas que sintetizam toxinas Bt, destacam-se interações dessas proteínas com células de mamíferos, bem como seus efeitos potencialmente tóxicos e alergênicos (*in vivo* e *in vitro*).

No caso das plantas TH, após breve revisão de artigos que observam efeitos negativos sobre a saúde humana e animal de herbicidas associados a esses cultivos, em especial aqueles à base de glifosato serão sistematizados estudos que apontam riscos e incertezas ligados ao consumo de tais plantas e suas partes. Além disso, algumas referências são fornecidas sobre riscos e incertezas para a saúde de plantas transgênicas não Bt e tolerantes a outros herbicidas além daqueles à base de glifosato.

Enfim, esse capítulo reúne estudos que apontam riscos relacionados à transferência horizontal de genes em células de mamíferos e seus organismos simbiontes, bem como incertezas associadas a mecanismos epigenéticos (RNAi em especial).

Na quinta e última parte deste livro, intitulada **Polêmicas científicas e críticas ao processo de análise do risco das plantas transgênicas**, analisa-se o crescente embate acadêmico e científico, levado a termo em escala internacional, quanto à suposta ausência de riscos relativos ao plantio e ao consumo de plantas transgênicas. Para tanto, estão sistematizados mais de 90 artigos que criticam desde o processo de avaliação de risco que indica inocuidade dessas plantas sob a perspectiva toxicológica, alergênica, nutricional e ambiental, até aqueles que apontam evidências de problemas nesse sentido, como riscos e danos toxicológicos, alergênicos,nutricionais e ambientais.

Campanhas de descredibilização de pesquisas e autores que mencionam tais riscos e incertezas são também ilustradas. O capítulo ainda inclui relatos diversos sobre temas que sugerem explicações para a ausência de consenso entre os cientistas, cobrindo desde conflitos de interesse e fragilidades metodológicas até comprometimentos com possíveis resultados da pesquisa e seus eventuais desdobramentos econômicos.

Aspectos Metodológicos

Artigos selecionados

Como anteriormente mencionado, o primeiro critério para a inclusão dos cerca de 750 artigos referenciados nesta publicação é sua publicação numa revista científica de renome nacional e/ou internacional – que em sua grande maioria possuem um comitê de releitura por pares (*peer-reviewed journals*). A publicação de um estudo em tais revistas permite de fato o seu acesso pela comunidade científica – que terá à disposição os materiais e a metodologia usados para sua condução, bem como parte significativa dos dados brutos, que não resultam da análise dos autores e que estão sujeitos a interpretações diversas. Nesse sentido, caso outros pesquisadores discordem da interpretação dos autores do estudo, esses últimos poderão exprimir suas opiniões divergentes, em geral por meio de um comunicado ou de outra publicação na mesma revista ou até em outra revista especializada no mesmo tema. Essa possibilidade de contra-argumentação para a comunidade científica não é possível quando se trata de relatórios internos de institutos, ONGs ou empresas, nos quais apenas os autores tiveram acesso aos dados.

Cabe destacar que o fato de um estudo ser publicado numa dessas revistas não significa a priori que os argumentos apresentados são os mais exatos, e menos ainda que as conclusões dos autores encerram algum tipo de verdade absoluta. Entretanto, na ausência de manifestação contrária por partes de membros da comunidade científica, tal estudo pode ser considerado o mais próximo de uma descrição verídica de determinada situação, num determinado momento. De fato, uma hipótese científica pode ser considerada como robusta até que a própria ciência comprove o contrário: é dessa forma que a ciência avança.

Além disso, o fato de os estudos serem revisados por pares antes da sua publicação minimiza a probabilidade de disponibilizar para a sociedade resultados que incorporam vieses metodológicos e conclusões não apoiadas pelos dados e suas implicações/interpretações.

Nesse sentido, a opção de selecionar apenas estudos e trabalhos publicados em revistas científicas que disponham de conselho editorial especializado e pratiquem revisão cega (os revisores não sabem quem são os autores e vice-versa) deve ser considerada como a melhor alternativa para cotejar opiniões independentes sobre qualquer assunto científico.

Categorias de artigos

Para auxiliar o leitor no uso desta publicação, adotamos no documento uma tipologia classificatória.

As referências são antecedidas por siglas que indicam a sua natureza. Assim, quando o artigo mencionado tem como objetivo anunciado uma revisão bibliográfica sobre determinado tema, a referência é precedida da sigla REV-; a sigla COM- diz respeito a "comunicações" ou "comentários" (geralmente caracterizados por um tempo de trabalho rápido e que ocupa uma ou duas páginas no máximo numa revista científica); a sigla MOD- indica um estudo que trata de uma modelização ou modelo experimental e a sigla EPI- diz respeito a estudo epidemiológico. A ausência de siglas indica artigos embasados por um experimento, com material e método explicitados, independentemente de serem realizados a campo ou em laboratório. Essa tipologia classificatória visa esclarecer o leitor sobre o tipo de referência mencionada, sendo em geral inapropriada uma comparação direta entre dois estudos que pertencem a categorias distintas.

Enfim, para fornecer uma visão histórica do tema, os artigos referenciados foram ordenados cronologicamente para cada problemática tratada. Além de facilitar a leitura, esse ordenamento salienta as raízes históricas do debate e ilustra a continuidade no tempo das polêmicas sobre os diversos temas tratados.

Acesso à informação e público-alvo

Cabe mencionar que a totalidade desses artigos estão disponíveis em meio digitais por meio da internet – parte significativa destes de forma gratuita (em especial via Open Access) -, dependendo da intenção dos autores e da opção dos editores das revistas, e o restante apenas via pagamento ou acesso por meio de servidores pré-pagos de universidades e instituições de pesquisa.

Para facilitar a consulta dos artigos, foram associados a cada referência de livre acesso, os links de internet que conduzem diretamente à publicação on-line. Quando o artigo não se encontra em sistema de livre acesso, o link associado direcionará para o resumo (*abstract*), sempre acessível on-line.

De modo geral, foram priorizados links de internet do Nacional Center for Biotechnology Information (NCBI – EUA). Diversos motivos orientaram essa opção, tais como o amplo banco de dados sobre estudos de biossegurança de plantas transgênicas, a presença da ferramenta *cross ref* – que permite identificar estudos relacionados, *corringendum* e respostas a um determinado artigo –, além do caráter público do órgão. Quando algum artigo não foi encontrado nesse banco de dados, foi indicado outro site de internet. Em todos os casos evitou-se priorizar, promover ou apoiar determinados sites ou instituições. Aquelas especializadas no comércio de publicação científica ou participantes de Organizações da Sociedade Civil receberam tratamento similar e secundário, conforme mencionamos acima. Em termos práticos, a facilidade de localizar publicações on-line por meio de ferramenta de busca na internet básica (Google) consistiu de certo modo em um critério que orientou significativamente a indicação dos links de internet usados nessa publicação. Evidentemente os artigos enquanto tal independem dessas indicações e podem ser acessados por outros caminhos.

Todos os links que constam nesta publicação foram consultados entre 1º/11/2014 e 30/3/2015.

Os resumos e as referências[6] aqui transcritos mantêm o idioma da publicação original. Pelo fato de o idioma inglês ser amplamente dominante na literatura científica, essa situação se repete neste livro. Entendemos que a opção se legitima pelas dificuldades técnicas envolvidas na tradução de um *abstract* de estudo científico já validado por pares, sem risco de alterações – ainda que leves – no sentido aportado pelos autores. Como exemplo, considere-se que, mesmo a interpretação de palavras simples, como "*might*" ou "*may*" (geralmente traduzidas ao português por "pode") são passíveis de implicar mudanças relevantes no sentido da frase original.

Embora considerando a importância da familiaridade com o inglês técnico para o aproveitamento adequado dos conteúdos aqui expostos, destacamos que este livro não se destina exclusivamente a

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[6] Pela opção adotada nesta publicação de manter as referências no idioma inglês, o sistema de normas da ABNT não pôde ser usado.

biotecnólogos, biólogos moleculares ou geneticistas. Ao contrário, considera-se necessário ampliar o debate a respeito dos riscos e das incertezas sobre o meio ambiente, a saúde e a produção agrícola das plantas transgênicas. E este livro pretende contribuir para tanto, ao disponibilizar estudos científicos sobre esses temas para outras esferas da comunidade científica. Nesse sentido o público-alvo inclui ecólogos, estatísticos, microbiologistas, nutricionistas, toxicologistas, veterinários, médicos, engenheiros agrônomos, agrícolas e florestais, entre outros. Entendemos que tais categorias profissionais estão debilmente representadas no debate científico e, consequentemente, na tomada de decisões associadas a esses temas, o que impacta decisivamente no exercício de suas profissões.

Este livro contribuirá para tanto. Nele se perceberá que, excluídos poucos resumos singularmente associados a determinadas especialidades do campo da biologia, os estudos aqui referenciados, bem como os textos que os introduzem, oferecem apoio robusto para formadores de opinião vinculados às diversas categorias profissionais já referidas, bem como a gestores públicos das áreas de saúde humana, animal e ambiental. O conteúdo também será de grande utilidade a legisladores, fiscais da lei e representantes da sociedade civil organizada – ainda que desprovidos de formação acadêmica em "ciências duras/exatas". Cabe mencionar que parte significativa dos termos técnicos estranhos aos não especialistas estará definida em notas de rodapé, ao longo do material. Em complemento, fornecemos no final do livro pequeno glossário explicativo que ajudará a dirimir dúvidas sobre expressões que, mesmo conhecidas do público em geral, podem assumir significados distintos no contexto da biologia.

Acadêmicos vinculados a ciências sociais e afins incluem-se também entre o público-alvo deste livro. Entendemos que poderão ampliar seus conhecimentos técnicos sobre o tema e enriquecê-los com expertises de sua área. Entendemos relevante a aproximação desses profissionais do debate sobre os OGMs e com a prática da análise e da tomada de decisão levadas a termo nas instâncias reguladoras, ante as diversas interpretações do Princípio da Precaução. Supomos que este documento poderá contribuir para a aquisição de conhecimentos sobre biologia e genética, e, em consequência, estimular a sua incorporação aos debates destas áreas científicas.

Esperamos também que este livro seja adotado como fonte de informação instrumentalizadora de profissionais e atores sociais que trabalham no tema dos agrotóxicos e dos seus impactos sobre o meio ambiente, a saúde pública e a produção agrícola.

Enfim, este trabalho incorpora, por meio dos artigos referenciados, gigantesco esforço internacional aplicado à construção de conhecimentos científicos, abrangendo diversos domínios da biologia e representando desafio inédito ao cidadão comum (potencialmente consumidor involuntário de OGM!), que aqui encontrará informações que permitirão melhor compreensão da tecnologia associada aos OGMs e de alguns mecanismos inerentes à vida, sob suas mais diversas formas e complexidades.

Boa leitura!

Gilles Ferment, Leonardo Melgarejo, Gabriel Fernandes e José Maria Ferraz.

Contextualização

1 Transgênicos e noções básicas de biologia

O processo de transgenia modifica o ser vivo, permitindo a fusão de material genético de espécies completamente diferentes, sejam bactérias, fungos, plantas ou animais. Para entender melhor como essa tecnologia funciona e quais riscos ela pode trazer para o meio ambiente e os consumidores, são indispensáveis algumas noções básicas de biologia.

1.1 Células, proteínas, genoma e regulação gênica

Qualquer ser vivo é constituído de uma ou várias células (organismo unicelular ou pluricelular). Em função da espécie, o numero de células pode variar enormemente: enquanto o ser humano possui aproximadamente $10E^{15}$ células, a *Caenorhabditis ele*gans (verme de uso frequente em pesquisa biológica) tem exatamente 1.031 células, e as bactérias têm só uma. De modo geral, o número de células de um organismo depende do seu tamanho/volume.

Nessas células existem vários componentes que realizam diversas funções essenciais àquelas “unidades da vida”, como as de reprodução (multiplicação celular), de comunicação (com outras células) e de respiração (celular), entre outras.

Nos organismos pluricelulares as células não possuem as mesmas funções, efetuando “tarefas” distintas. As células do fígado terão função importante na desintoxicação de moléculas nocivas e as células do sistema imunológico (glóbulos brancos, por exemplo) terão papéis biológicos mais amplos, relacionados à proteção do organismo contra infecções virais ou bacterianas, por exemplo. No caso das plantas, as células da raiz terão funções diferentes das células dos grãos ou das folhas, que ainda apresentarão diferenciações associadas aos sistemas de que participam.

Mas, independentemente das funções envolvidas (sejam comuns ou específicas a determinadas células), a quase totalidade das ações celulares serão efetuadas por intermédio das proteínas. As proteínas correspondem, nesse sentido, às principais moléculas funcionais das células. Algumas proteínas transportarão materiais para fora ou para dentro da célula, outras ajudarão a destruir ou recortar elementos velhos ou perigosos, outras atuarão na produção de energia ou na multiplicação celular, e assim por diante.

1.2 De onde vem a diversidade de proteínas e como elas são produzidas para desenvolver determinadas e tão distintas funções?

Parte das respostas está localizada no núcleo de cada célula. Nesse compartimento existem macromoléculas que contêm a informação necessária para o funcionamento da célula (ou do organismo unicelular): são as famosas fitas de DNA[7].

Concretamente, uma fita de DNA poderá ser constituída por bilhões de moléculas de Ácido Desoxirribonucleico (DNA). A variação dependerá da espécie de organismo que carrega aquele DNA. Existem quatro tipos de bases nitrogenadas (Adenina, Timina, Guanina e Citosina), designadas pelas letras A, T, G e C.

A complexidade do DNA pode ser ilustrada pela seguinte estimativa: a transcrição simples da informação "escrita" dentro do núcleo de uma única célula humana, usando aquelas quatro letras, exigiria a publicação de 10 livros, em formato A4, com 1.000 páginas cada.

Determinadas sequências de Ácidos Desoxirribonucleicos possuem significações particulares. Como no caso das várias letras do nosso alfabeto, que quando associadas em determinada ordem geram frases com sentido próprio, a ordem na qual aquelas quatro bases se sucedem define o que podemos chamar de mensagens biológicas.

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[7] Durante o processo de divisão celular, principalmente, essas fitas de DNA são aglomeradas e compactadas e podem ser visualizadas na forma de cromossomos.

A ciência designou essas mensagens biológicas (códigos) de genes. O conhecimento atual evoluiu e hoje já se sabe que os genes sozinhos não são capazes de comandar determinadas expressões , pois é importante a sua relação com o meio, além de existirem sequências de bases nitrogenadas que não formam genes, mas atuam como elementos de regulação fundamentais para que aquelas mensagens sejam emitidas, lidas ou compreendidas. Em outras palavras, menos da metade da base genética é formada por genes. O restante, que, por não determinar a formação de proteínas, inicialmente foi interpretado como “DNA lixo”, possui funções regulatórias indispensáveis ao funcionamento dos genes. É nesse sentido que a interpretação inicial de que os genes definem a vida como ela é, caiu por terra, sendo admitido no presente que não se sabe exatamente o que seja um gene.

Mas é por causa daquela interpretação reducionista que se costuma dizer, que o DNA, mediante seu conjunto de genes, constitui uma espécie de “livro da vida”, onde se esconde o suporte da informação genética e todas suas possibilidades.

Normalmente existem vários milhares de genes em cada molécula de DNA. Alguns organismos são mais complexos que outros e o homem, que possui aproximativamente 22 mil genes, não está no topo da lista. O arroz, por exemplo, possui cerca de 50 mil genes.

Em todos os organismos é a mensagem codificada no DNA, com apoio das sequências reguladoras e submetida a pressões e particularidades do meio celular, que orienta a elaboração de proteínas, estimulando a ampla variedade de funções e características de todos os organismos vivos. Assim, aquelas combinações de bases nitrogenadas são cruciais para formação de vastíssimo conjunto de moléculas que dá base a todos os processos biológicos conhecidos e imagináveis.

De forma simplificada assume-se que um gene sempre é traduzido em proteína(s). Mas cabe mencionar que a informação genética contida na fita de DNA não pode ser traduzida diretamente em proteínas. Ela precisa passar por um processo de transcrição, "reescritura ou recodificação". Esse processo permite ao DNA ser copiado (transcrito) numa outra molécula – a fita de Ácido Ribonucleico qualificada de mensageira (RNAm) – antes de servir de base à elaboração de proteínas. Em outras palavras, é no processo chamado de transcrição que o RNAm será traduzido em aminoácidos, que por sua vez formarão um ou mais tipos de proteínas.

1.3 Regulação gênica

Resumindo, a fita de DNA é constituída de genes que contêm a informação genética necessária para a fabricação de proteínas responsáveis por quase todos os processos biológicos realizados pelas células (salvo vírus e outras exceções). Além disso, todas as células de um determinado organismo terão a mesma molécula de DNA e, portanto, a mesma informação genética. Mas, nem por isso essa informação se expressará da mesma maneira em todas as células. Essa constatação introduz o conceito de regulação gênica ou regulação da expressão gênica.

De fato, em qualquer momento ao longo da história de vida de uma célula, apenas uma pequena parcela do RNA e proteínas codificadas em seu genoma serão expressas. Em momentos diferentes, o perfil de produtos gênicos expressos pode diferir marcantemente em termos qualitativos e quantitativos com relação a quais proteínas serão expressas e em que nível de expressão).

Qualquer passo da expressão de um gene pode ser modulado, desde a acessibilidade[8] do DNA para a sua transcrição até a modificação pós-translacional[9] de uma proteína, passando pela quantidade de transcritos de RNA sintetizados. A regulação gênica assegura à célula parte significativa do controle sobre a sua estrutura e função, sendo a

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[8] De modo geral, o DNA é acessível à maquinaria transcricional apenas quando não compactado, numa forma denominada de eucromatina.

[9] Processos biológicos que visam finalizar a síntese de uma proteína após o processo de tradução de fitas de RNA para cadeias de aminoácidos, adicionando, por exemplo, determinadas moléculas (açúcares, fósforos) ou conferindo-lhe uma conformação espacial tridimensional específica (via dobramento e fixação bioquímica).

base de mecanismos biológicos extremamente diversos como a diferenciação celular, a morfogênese ou a adaptabilidade de qualquer organismo ao ambiente.

Outra parte regulatória da expressão gênica é controlada pelo ambiente onde o organismo ou a célula se desenvolve. A epigenética trata do estudo das influências ambientais, que modificam a expressão do código genético e dos mecanismos ali envolvidos[10]. Os mecanismos epigenéticos, brevemente retomados nesta publicação (em especial na parte 1), são diversos e podem atuar também em vários momentos da elaboração de proteínas. Além disso, os efeitos de mecanismos epigenéticos podem ser transmissíveis de geração a geração, embora sua maioria pareça ser reversível no tempo, enquanto as alterações do DNA (como mutações) são definitivas.

Os caracteres observados (como as estruturas físicas que diferenciam os diversos tipos de célula) são denominados fenotípicos. Na escala de um organismo, a cor dos olhos, a altura de inserção da espiga, o tamanho ou a capacidade de resistência a agentes patógenos seriam exemplos de expressões fenotípicas. Assim, o fenótipo de um organismo (ou de uma célula) resultará de determinado tipo de expressão do genótipo, que emergirá de vasto conjunto de possibilidades, sendo parcialmente controlado pelo ambiente.

2 Organismos geneticamente modificados e plantas transgênicas alimentares: debates distintos

Por meio de ferramentas de biologia molecular, pesquisadores conseguem transferir determinadas sequências de DNA de um organismo para o genoma de outro, visando conferir a este último uma nova função biológica, supostamente no interesse da sociedade. Teoricamente, qualquer ser vivo pode ser transformado em laboratório em

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[10] O campo da epigenética tem sido amplamente desconsiderado (quando não simplesmente ignorado) na avaliação de risco de OGMs. Possivelmente porque confronta paradigmas e dogmas ultrapassados, mas que ainda fundamentam parte significativa da biologia molecular e da genética, reativando polêmicas históricas tal como a da transmissão de caracteres adquiridos (teoria de Lamarck) ou, mais recentemente, a corrida em busca de tecnologias ajustadas ao campo financeiramente promissor da terapia gênica. A desconsideração aos conhecimentos da epigenética claramente reduz a eficácia das avaliações de risco e retarda o progresso científico.

um organismo transgênico. Os exemplos atuais incluem plantas, bactérias, leveduras, camundongos, peixes, ovelhas e cabras entre outros. Alguns desses têm se revelado ferramentas de pesquisa muito pertinentes e interessantes para o entendimento e a construção do conhecimento científico sobre conceitos e mecanismos genéticos e de regulação gênica. Outros, nem tanto.

De fato, as biotecnologias são usadas, há várias décadas, tanto no campo da pesquisa quanto no suporte/auxílio a processos químico-industriais em escala comercial. Assim é com a produção de insulina em OGM, de proteínas antivirais (para fabricação de vacinas) ou de enzimas com propriedades detergentes, entre outras. Entretanto, o comércio desses produtos – produzidos em OGM – não galvanizou o debate sobre biotecnologias com a mesma intensidade que as plantas transgênicas destinadas à alimentação. Vários fatores, sociais e biológicos, permitem explicar essa diferenciação. De modo sintético, destacam-se os seguintes argumentos:

a) no caso das plantas transgênicas alimentares, a saúde dos consumidores é diretamente envolvida, afetando todas as pessoas. No caso de produtos medicinais como a insulina ou vacinas recombinantes, apenas determinadas populações são envolvidas, as quais tem a oportunidade de optar, de modo geral, conscientemente por isso. Além disso, essas populações são protegidas por mecanismos sociais e institucionais que permitem um monitoramento relativamente eficiente dos problemas potenciais, pois existem receitas médicas e acompanhamento caso a caso, por exemplo;

b) ao se consumirem partes de uma planta transgênica, é o organismo geneticamente modificado em si que está sendo ingerido, e não apenas um produto de expressão de OGM – tal como uma enzima produzida em levedura transgênica e subsequentemente purificada antes de ser integrada na cadeia alimentar. Tanto do ponto de vista biológico como relativamente aos riscos para a saúde, isso implica diferenças marcantes. O uso, após processo de purificação de apenas uma proteína produzida em OGM, não se estende a todo um conjunto de possibilidades de danos associados a eventuais modificações genéticas e/ou alterações metabólicas que podem ocorrer na escala do organismo inteiro. Tal conjunto de possibilidades, ao contrário, estão presentes na situação em que se consome o OGM propriamente dito, ou parte dele.

c) As plantas transgênicas comerciais são cultivadas em ambientes não controlados, ou seja, no campo. Já os OGMs desenvolvidos com objetivos experimentais e/ou destinados à produção de moléculas recombinantes para usos industriais diversos são confinados a ambientes "fechados", mesmo se não necessariamente herméticos, tais como laboratórios, incubadores, câmara de vegetação etc. Esse fato limita substancialmente os riscos associados ao consumo de tais produtos transgênicos por organismos não alvo (ONA), bem como os riscos associados à disseminação de transgenes no meio ambiente e seus impactos socioambientais.

Essas observações não devem ser interpretadas como argumentação que isente as biotecnologias não agrícolas/alimentares de qualquer risco biológico. Permitem, contudo, delimitar claramente o quadro da polêmica científica tratada nesta publicação.

Este livro é claro em seu esforço de discriminação entre os debates científicos relativos aos riscos da biotecnologia em geral e os riscos associados ao uso comercial de plantas transgênicas alimentares. "Argumentos" do tipo "transgênicos são bons para as nossas sociedades porque já salvaram milhões de vidas de pessoas insulino-dependentes[11]" – além de não possuírem base científica – não cabem numa reflexão séria sobre os problemas aqui discutidos.

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[11] Esse tipo de argumento falacioso, que pretende silenciar o debate sobre os riscos reais para a saúde pública, o meio ambiente e a agricultura, associados ao uso comercial de plantas transgênicas alimentares, tem sido, infelizmente, repetido por parte expressiva do mundo acadêmico e sociedades científicas do Brasil e de outros países grandes produtores de plantas transgênicas.

3 Plantas transgênicas e avaliação de risco: contexto internacional e brasileiro

Hoje, no cenário mundial, mais de 98% das plantas transgênicas foram geneticamente modificadas para expressar apenas dois tipos de características:

a) a síntese de uma toxina inseticida nos seus tecidos: são as chamadas plantas Bt, que teoricamente são "resistentes" a insetos, pois o inseto alvo, via de regra uma lagarta ou uma larva de coleóptero, ao se alimentar da folha de milho, soja ou algodão ou da raiz (principalmente de milho Bt), irá ingerir aquelas toxinas e morrerá.

b) a modificação de vias metabólicas nas plantas que lhes permitem ser insensíveis à ação letal de determinados herbicidas: essas plantas são denominadas pela indústria de biotecnologia de plantas Tolerantes a Herbicidas (TH). O exemplo mais conhecido é representado pela soja RR, que ao receber um banho de glifosato não morre, contrariamente a todas as outras plantas da lavoura tratada com o agrotóxico, facilitando assim o manejo das plantas ruderais nessas lavouras. Além da tolerância ao glifosato, plantas com função de tolerância a herbicidas à base de glufosinato de amônio, de 2,4-D ou do grupo químicos das imidazolinonas são hoje cultivadas em escala comercial.

Nesses últimos anos, cada vez mais áreas são plantadas com plantas transgênicas que combinam[12] essas duas funções, ou seja, são plantas tóxicas a determinados insetos e também insensíveis a determinados herbicidas.

As plantas transgênicas são basicamente produzidas em apenas cinco países no mundo (EUA, Brasil, Argentina, Índia[13] e Canadá), totalizando cerca de 95% dos 180 milhões de hectares cultivados no planeta.

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[12] Essa combinação de funções pode ser obtida diretamente por transgenia, transferindo na mesma planta vários transgenes responsáveis por funções diferentes ou por cruzamento convencional de duas ou mais plantas transgênicas que possuem essas funções isoladamente. Nesse caso, trata-se de eventos piramidados/estaqueados.

[13] Na Índia apenas o algodão transgênico é autorizado na escala comercial, o que significa que variedades transgênicas voltadas ao consumo humano e animal não são cultivadas.

Cabe mencionar que basicamente seis empresas detêm o mercado mundial de variedades transgênicas e dos agrotóxicos a elas associados[14]. Isso se aplica tanto ao cenário nacional como internacional.

Dois principais tipos de riscos podem ser associados ao uso de OGMs:

a) riscos associados à nova função conferida por meio da transgenia, a proteína inseticida sintetizada em plantas Bt, por exemplo e à presença do(s) transgene(s) associado(s);

b) riscos associados a efeitos não desejados resultantes do próprio processo de transgenia, como a alteração de vias metabólicas que podem resultar na síntese de novas proteínas, potencialmente tóxicas ou alergênicas.

Essa distinção entre tipo de riscos é observada, na maior parte deste livro, graças à elaboração de itens referentes a riscos específicos. Todos os riscos biológicos associados ao uso comercial de plantas transgênicas decorrem basicamente desses dois tipos de riscos. Incluem-se aqui os riscos associados à disseminação transgênica no meio ambiente ou ao consumo dessas plantas por organismos não alvo, animais e seres humanos.

Apesar desta publicação não cobrir apenas riscos específicos ao contexto brasileiro, a quase totalidade dos artigos referenciados alertam para riscos e incertezas relevantes para o Brasil.

De fato, o Brasil é o segundo maior produtor de plantas transgênicas alimentares no mundo, com cerca de 30 milhões de hectares plantados com soja, milho e algodão transgênicos. Não cultivados ainda, mas que já obtiveram a aprovação da CTNBio, temos ainda um feijão e um eucalipto. Por isso, a totalidade da área plantada com

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[14] Exemplificando, a Monsanto é responsável pela comercialização das principais variedades de soja, milho e algodão tolerantes ao glifosato e detém também as principais patentes sobre herbicidas à base de glifosato; a Bayer é responsável pela comercialização das principais variedades de soja, milho e algodão tolerantes ao glufosinato de amônio e detém também as principais patentes sobre herbicidas à base de glufosinato de amônio; a DowAgroscience é responsável pela comercialização das principais variedades de soja e milho tolerantes ao 2,4-D e detém também as principais patentes de herbicidas a base de 2,4-D, etc.

transgênicos no país é coberta por esses dois tipos de transgênicos, variedades Bt ou TH ou que combinam essas duas funções.

A avaliação do risco no Brasil é coordenada[15] pela Comissão Técnica Nacional de Biossegurança (CTNBio), comissão do MCTI composta por 27 doutores, incluindo representantes do governo, da academia e da sociedade civil. Em 10 anos de existência, a CTNBio aprovou cerca de 60 eventos transgênicos (45 plantas). Jamais houve rejeição aos pedidos das empresas. Ora, todas as decisões da CTNBio foram polêmicas desde sua instituição, em 2005. Como transparece ao longo desta publicação, a opinião técnica da CTNBio não reflete a opinião da comunidade científica.

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[15] Teoricamente subordinadas à análise socioeconômica do Conselho Nacional de Biossegurança (CNBS, composto por onze ministros de Estado) – as opiniões técnicas emitidas pela CTNBio são na verdade transformadas em decisões políticas que resultam em autorização de comercialização de variedades transgênicas em tempo recorde. Na ausência de manifestação do CNBS – como é o caso para 95% das plantas transgênicas hoje autorizadas – é a CTNBio que é moralmente (mas não legalmente) responsável por parte significativa da política agrícola do país, fortemente voltada ao uso da transgenia.

Parte 1

Efeitos imprevisíveis e não intencionais da transgenia

1. Genoma, epigenoma e expressão gênica[16]

A transgenia é uma ferramenta de biologia molecular que tem como objetivo transferir determinados genes de um organismo para outro, visando transferir também características supostamente dependentes daqueles genes. Entretanto, ao se tratar de produtos que serão liberados no meio ambiente e consumidos por seres humanos, é necessária enorme cautela quanto à totalidade de implicações e suas possíveis consequências.

Essas transferências gênicas incorporam, obviamente, alterações potencialmente indesejáveis, capazes de resultar em efeitos perigosos para a saúde e para o meio ambiente. Os controles que permitiriam atuar com os cuidados requeridos exigem, evidentemente, compreensão adequada das implicações associadas ao genoma e suas relações com o ambiente. O ponto mais fundamental, nessa discussão reside na dificuldade de, com os conhecimentos atuais, compreender corretamente o funcionamento do genoma e das alterações a ele impostas.

1.1 O conceito de gene em constante evolução

Os organismos transgênicos foram desenvolvidos com base em premissas exageradamente simplificadas, da biologia molecular e da genética. Segundo estas, um gene codifica para determinada proteína que desempenhará determinada função. Isso e mais nada. Nesse sentido, acreditava-se que bastaria simplesmente transferir um gene – com função interessante – desde um organismo (doador) até outro organismo (receptor) para que este último se visse obrigado a expressar a função desejada, por meio da síntese da proteína recombinante.

Ora, o raciocínio se tornou complexo quando o conceito de gene evoluiu, sendo hoje mais aceito o nome de "sequência genômica de tamanho determinado" ou ainda de "elementos de DNA", que ope-

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[16] Embora a maioria das referências mencionadas nesse item diga respeito à biologia em células de mamíferos (ou organismos modelos, tais como determinadas leveduras), cabe destacar que incorpora dúvidas válidas para organismos supostamente mais simples, incluindo os vegetais (foco desta publicação).

ram de forma articulada, condicionados pelo ambiente (ao qual afetam, enquanto são por ele afetados).

No caso do ser humano, aquela interpretação simplificada permitiu que a maior parte do genoma viesse a ser considerado, até alguns anos atrás, como “DNA lixo”, já que não seria codificador de proteína. Posteriormente, o projeto Encode concluiu (em 2012) que 80% do genoma teriam na verdade diversas funções bioquímicas reguladoras de sistemas complexos não previsíveis com base no conhecimento dominado pela ciência.

A interpretação se mantém. A rigor, não sabemos o que é um gene, embora saibamos que não se trata do que supúnhamos. Nesse sentido, é possível afirmar que o entendimento dos mecanismos biológicos básicos inerentes à vida carece de mais estudos para esclarecer seus modos de ação e oferecer segurança a suas manipulações. Os estudos a seguir discutem essa questão.

REV – El-Hani, C. 2007. Between the cross and the sword: the crisis of the gene concept. *Genet. Mol. Biol.,* vol.30, nº 2. São Paulo.

Challenges to the gene concept have shown the difficulty of preserving the classical molecular concept, according to which a gene is a stretch of DNA encoding a functional product (polypeptide or RNA). The main difficulties are related to the overlaying of the Mendelian idea of the gene as a ‘unit’: the interpretation of genes as structural and/or functional units in the genome is challenged by evidence showing the complexity and diversity of genomic organization. This paper discusses the difficulties faced by the classical molecular concept and addresses alternatives to it. Among the alternatives, it considers distinctions between different gene concepts, such as that between the ‘molecular’ and the ‘evolutionary’ gene, or between ‘gene-P’ (the gene as determinant of phenotypic differences) and ‘gene-D’ (the gene as developmental resource). It also addresses the process molecular gene concept, according to which genes are understood as the whole molecular process underlying the capacity to express a particular product, rather than as entities in ‘bare’ DNA; a treatment of genes as sets of domains (exons, introns, promoters, enhancers, etc.) in DNA; and a systemic understanding of genes as combinations of nucleic acid sequences corresponding to a product specified or demarcated by the cellular system. In all these cases, possible contributions to the advancement of our understanding of the architecture and dynamics of the genetic material are emphasized.

Artigo completo disponível em http://www.scielo.br/scielo.php?pid=S1415-47572007000300001&script=sci_arttext

Encode Project Consortium. 2007. Identification and analysis of functional elements in 1% of the human genome by the Encode pilot project. *Nature*, v.447, p. 799-816.

We report the generation and analysis of functional data from multiple, diverse experiments performed on a targeted 1% of the human genome as part of the pilot phase of the ENCODE Project. These data have been further integrated and augmented by a number of evolutionary and computational analyses. Together, our results advance the collective knowledge about human genome function in several major areas. First, our studies provide convincing evidence that the genome is pervasively transcribed, such that the majority of its bases can be found in primary transcripts, including non-protein-coding transcripts, and those that extensively overlap one another. Second, systematic examination of transcriptional regulation has yielded new understanding about transcription start sites, including their relationship to specific regulatory sequences and features of chromatin accessibility and histone modification. Third, a more sophisticated view of chromatin structure has emerged, including its inter-relationship with DNA replication and transcriptional regulation. Finally, integration of these new sources of information, in particular with respect to mammalian evolution based on inter- and intra-species sequence comparisons, has yielded new mechanistic and evolutionary insights concerning the functional landscape of the human genome. Together, these studies are defining a path for pursuit of a more comprehensive characterization of human genome function.

http://www.ncbi.nlm.nih.gov/pubmed/17571346

Encode Project Consortium. 2012. An integrated encyclopedia of DNA elements in the human genome. *Nature*, Vol 489, 57-74.

The human genome encodes the blueprint of life, but the function of the vast majority of its nearly three billion bases is unknown. The Encyclopedia of DNA Elements (ENCODE) project has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification. These data enabled us to assign biochemical functions for 80% of the genome, in particular outside of the well-studied protein-coding regions. Many discovered candidate regulatory elements are physically associated with one another and with expressed genes, providing new insights into the mechanisms of gene regulation. The newly identified elements also show a statistical correspondence to sequence variants linked to human disease, and can thereby guide interpretation of this variation. Overall, the project provides new insights into the organization and regulation of our genes and genome, and is an expansive resource of functional annotations for biomedical research.

http://www.ncbi.nlm.nih.gov/pubmed/22955616

COM – Pennisi, E. 2012. Encode Project Writes Eulogy for Junk DNA. *Science*, 1159-1161.

Sem resumo disponível.

http://www.ncbi.nlm.nih.gov/pubmed/22955811

Ibarra-Laclette, E.; Lyons, E.; *et al*. 2013. Architecture and evolution of a minute plant genome. *Nature,* 498, 94-98 doi:10.1038/nature12132.

It has been argued that the evolution of plant genome size is principally unidirectional and increasing owing to the varied action of whole-genome duplications (WGDs) and mobile element proliferation. However, extreme genome size reductions have been reported in the angiosperm family tree. Here we report the sequence of the 82-megabase genome of the carnivorous bladderwort plant Utricularia gibba. Despite its tiny size, the U. gibba genome accommodates a typical number of genes for a plant, with the main difference from other plant genomes arising from a drastic reduction in non-genic DNA. Unexpectedly, we identified at least three rounds of WGD in U. gibba since common ancestry with tomato (Solanum) and grape (Vitis). The compressed architecture of the U. gibba genome indicates that a small fraction of intergenic DNA, with few or no active retrotransposons, is sufficient to regulate and integrate all the processes required for the development and reproduction of a complex organism.

http://www.ncbi.nlm.nih.gov/pubmed/23665961

Ainda hoje, várias questões permanecem abertas, relativamente aos papéis biológicos desempenhados pela maior parte dos elementos de DNA, à forma como estes se rearranjam no genoma, como são aceitos ou rejeitados quando alterados/quebrados por processos naturais ou artificiais.

Sargent, R.; Brenneman, M.; Wilson, J. 1997. Repair of Site-Specific Double-Strand Breaks in a Mammalian Chromosome by Homologous and Illegitimate Recombination. *Molecular and Cellular Biology*, p. 267–277.

In mammalian cells, chromosomal double-strand breaks are efficiently repaired, yet little is known about the relative contributions of homologous recombination and illegitimate recombination in the repair process. In this study, we used a loss-of-function assay to assess the repair of double-strand breaks by homologous and illegitimate recombination. We have used a hamster cell line engineered by gene targeting to contain a tandem duplication of the native adenine phosphoribosyltransferase (APRT) gene with an I-SceI recognition site in the otherwise wild-type APRT+ copy of the gene. Site-specific double-strand breaks were induced by intracellular expression of I-SceI, a rare-cutting endonuclease from the yeast Saccharomyces cerevisiae. I-SceI cleavage stimulated homologous recombination about 100-fold; however, illegitimate recombination was stimulated more than 1,000-fold. These results suggest that illegitimate recombination is an important competing pathway with homologous recombination for chromosomal double-strand break repair in mammalian cells.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC231751/

Haber, J. 2006. Transpositions and translocations induced by site-specific double-strand breaks in budding yeast. *DNA Repair (Amst)*, 5(9-10):998-1009.

Much of what we know about the molecular mechanisms of repairing a broken chromosome has come from the analysis of site-specific double-strand breaks (DSBs). Such DSBs can be generated by conditional expression of meganucleases such as HO or I-SceI or by the excision of a DNA transposable element. The synchronous creation of DSBs in nearly all cells of the population has made it possible to observe the progress of recombination by monitoring both the DNA itself and proteins that become associated with the recombining DNA. Both homologous recombination mechanisms and non-homologous end-joining (NHEJ) mechanisms of recombination have been defined by using these approaches. Here I focus on recombination events that lead to alterations of chromosome structure: transpositions, translocations, deletions, DNA fragment capture and other small insertions. These rearrangements can occur from ectopic gene conversions accompanied by crossing-over, break-induced replication, single-strand annealing or non-homologous end-joining.

http://www.ncbi.nlm.nih.gov/pubmed/16807137

Katz, S.; Gimble, F.; Storici, F. 2014. To nick or not to nick: comparison of I-SceI single- and double-strand break-induced recombination in yeast and human cells. *PLOS ONE*, 9(2):e88840. doi: 10.1371/journal.pone.0088840.

Genetic modification of a chromosomal locus to replace an existing dysfunctional allele with a corrected sequence can be accomplished through targeted gene correction using the cell's homologous recombination (HR) machinery. Gene targeting is stimulated by generation of a DNA double-strand break (DSB) at or near the site of correction, but repair of the break via non-homologous end-joining without using the homologous template can lead to deleterious genomic changes such as in/del mutations, or chromosomal rearrangements. By contrast, generation of a DNA single-strand break (SSB), or nick, can stimulate gene correction without the problems of DSB repair because the uncut DNA strand acts as a template to permit healing without alteration of genetic material. Here, we examine the ability of a nicking variant of the I-SceI endonuclease (K223I I-SceI) to stimulate gene targeting in yeast Saccharomyces cerevisiae and in human embryonic kidney (HEK-293) cells. K223I I-SceI is proficient in both yeast and human cells and promotes gene correction up to 12-fold. We show that K223I I-SceI-driven recombination follows a different mechanism than wild-type I-SceI-driven recombination, thus indicating that the initial DNA break that stimulates recombination is not a low-level DSB but a nick. We also demonstrate that K223I I-SceI efficiently elevates gene targeting at loci distant from the break site in yeast cells. These findings establish the capability of the I-SceI nickase to enhance recombination in yeast and human cells, strengthening the notion that nicking enzymes could be effective tools in gene correction strategies for applications in molecular biology, biotechnology, and gene therapy.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3928301/

Assim como o conceito de gene permanece em plena evolução, também o conhecimento científico a respeito do conjunto de mecanismos biológicos que permitem a regulação desses genes se amplia a cada ano. De fato, além dos promotores e das sequências reguladoras de DNA codificador, existem várias outras moléculas que possuem papel importante na regulação e na expressão gênica e que não são cobertas pelas avaliações de riscos em plantas transgênicas. Concentradas no estrito espaço dos promotores, dos reguladores e dos terminadores, tais análises, como realizadas atualmente, deixam de lado vasto campo para expressão de possibilidade de risco.

Desde há cerca de 20 anos, novas moléculas vêm sendo identificadas e caracterizadas como elementos reguladores da expressão gênica. São moléculas de RNA que não são traduzidas em proteínas, hoje denominadas de *small RNA species* (ou RNA não codificadores/*non-coding* RNA/ncRNA[17] ou ainda RNA funcional/fRNA).

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[17] Essa terminologia pode se mostrar equivocada, considerando que foi recentemente descoberta a possibilidade de determinadas *small RNA* species codificarem por peptídeos. Ver, por exemplo, Lauressergues et al., 2015 (Primary transcripts of microRNAs encode regulatory peptides, Nature, 520,90–93) e Waterhouse & Hellens, 2015 (Plant biology: Coding in non-coding RNAs Nature, 520,41–42).

Os sRNAs agrupam vários tipos de RNA[18], com ações diversas na regulação gênica, mas também na replicação do DNA ou em modificações pós-translacionais. No presente contexto, referente a possíveis alterações de sRNAs (e suas consequências) ao submeter um organismo/uma célula ao processo de transgenia, os miRNA e siRNA merecem destaque. Ambos possuem papel central no mecanismo hoje chamado de RNA interferência (RNAi), que determina a inibição da regulação gênica (em geral destruindo RNA mensageiros específicos – impedindo a formação de proteínas a partir destes).

O potencial biológico desse mecanismo, bem como das moléculas associadas, é enorme, apesar de ainda cheio de incertezas. De fato, um único miRNA pode reduzir o nível de expressão de centenas de genes. Essas moléculas possuem também capacidade de interagir na forma da cromatina da célula, deixando o DNA num estado físico incompatível com a sua transcrição em RNAm.

Em paralelo, mudanças químicas – principalmente concretizadas pela adição de grupos metil nos histonas e/ou no próprio DNA, por meio de metilases – também alteram a disponibilidade do gene para sua transcrição ou regulam diretamente a expressão do gene metilado, respectivamente.

Essas formas de regulação gênica basicamente independentes das sequências de DNA do organismo são em grande parte associadas a mecanismos epigenéticos, constituindo campo de acúmulo recente que não tem merecido cuidados compatíveis com sua relevância, no tocante à análise do risco das plantas transgênicas. De fato, essa análise focaliza hoje apenas sobre possíveis alterações do DNA do organismo transformado.

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[18] Ver http://en.wikipedia.org/wiki/List_of_RNAs para mais detalhes.

Fire A.; Xu S.; *et al*. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans. Nature,* 391(6669):806-11.

Experimental introduction of RNA into cells can be used in certain biological systems to interfere with the function of an endogenous gene. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode Caenorhabditis elegans to manipulate gene expression. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous mRNA and suggesting that there could be a catalytic or amplification component in the interference process.

Artigo completo disponível em http://www.nature.com/nature/journal/v391/n6669/full/391806a0.html

REV – Mello C.; Conte Jr D. 2004. Revealing the world of RNA interference. *Nature*, 432:338–42.

The recent discoveries of RNA interference and related RNA silencing pathways have revolutionized our understanding of gene regulation. RNA interference has been used as a research tool to control the expression of specific genes in numerous experimental organisms and has potential as a therapeutic strategy to reduce the expression of problem genes. At the heart of RNA interference lies a remarkable RNA processing mechanism that is now known to underlie many distinct biological phenomena.

http://www.ncbi.nlm.nih.gov/pubmed/15372040

Grewal, S.; Elgin, S. 2007. Transcription and RNA interference in the formation of heterochromatin. *Nature*, 447:399–406.

Recent findings have challenged the longstanding belief that heterochromatin is an inert and transcriptionally inactive structure. Studies in organisms ranging from fission yeast to animals have found that noncoding RNAs transcribed from heterochromatic DNA repeats function in the assembly and function of heterochromatin. In this review, we discuss the roles of RNA and RNA turnover in mechanisms that mediate heterochromatin assembly and keep heterochromatic domains silent.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2950806/

Washietl, S., Pedersen, J.; Korbel, Jan; *et al.* 2007. Structured RNAs in the Encode selected regions of the human genome. *Genome Research*, 17(6): 852–864.

Functional RNA structures play an important role both in the context of noncoding RNA transcripts as well as regulatory elements in mRNAs. Here we present a computational study to detect functional RNA structures within the ENCODE regions of the human genome. Since structural RNAs in general lack characteristic signals in primary sequence, comparative approaches evaluating evolutionary conservation of structures are most promising. We have used three recently introduced programs based on either phylogenetic–stochastic context-free grammar (EvoFold) or energy directed folding (RNAz and AlifoldZ), yielding several thousand candidate structures (corresponding to ~2.7% of the ENCODE regions). EvoFold has its highest sensitivity in highly conserved and relatively AU-rich regions, while RNAz favors slightly GC-rich regions, resulting in a relatively small overlap between methods. Comparison with the GENCODE annotation points to functional RNAs in all genomic contexts, with a slightly increased density in 3'-UTRs. While we estimate a significant false discovery rate of ~50%–70% many of the predictions can be further substantiated by additional criteria: 248 loci are predicted by both RNAz and EvoFold, and an additional 239 RNAz or EvoFold predictions are supported by the (more stringent) AlifoldZ algorithm. Five hundred seventy RNAz structure predictions fall into regions that show signs of selection pressure also on the sequence level (i.e., conserved elements). More than 700 predictions overlap with noncoding transcripts detected by oligonucleotide tiling arrays. One hundred seventy-five selected candidates were tested by RT-PCR in six tissues, and expression could be verified in 43 cases (24.6%).

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1891344/

Reyes-Turcu, F.; Grewal S. 2012. Different means, same end — heterochromatin formation by RNAi and RNAi-independent RNA processing factors in fission yeast. *Curr Opin Genet* Dev, 22:156–63.

The assembly of heterochromatin in eukaryotic genomes is critical for diverse chromosomal events including regulation of gene expression, silencing of repetitive DNA elements, proper segregation of chromosomes and maintenance of genomic integrity. Previous studies have shown that noncoding RNAs and the RNA interference (RNAi) machinery promote the assembly of heterochromatin that serves as a multipurpose platform for targeting effectors involved in various chromosomal processes. Recent work has revealed that RNAi-independent mechanisms, involving RNA processing activities that utilize both noncoding and coding RNAs, operate in the assembly of heterochromatin. These findings have established that, in addition to coding for proteins, mRNAs also function as signaling molecules that modify chromatin structure by targeting heterochromatin assembly factors.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3331891/

Ahlenstiel C.; Lim, H.; Cooper, D; Ishida, T.; Kelleher, A.; Suzuki, K. 2012. Direct evidence of nuclear Argonaute distribution during transcriptional silencing links the actin cytoskeleton to nuclear RNAi machinery in human cells. *Nucl Acids Res,* 40: 1579–95.

Mammalian RNAi machinery facilitating transcriptional gene silencing (TGS) is the RNA-induced transcriptional gene silencing-like (RITS-like) complex, comprising of Argonaute (Ago) and small interfering RNA (siRNA) components. We have previously demonstrated promoter-targeted siRNA induce TGS in human immunodeficiency virus type-1 (HIV-1) and simian immunodeficiency virus (SIV), which profoundly suppresses retrovirus replication via heterochromatin formation and histone methylation. Here, we examine subcellular co-localization of Ago proteins with promoter-targeted siRNAs during TGS of SIV and HIV-1 infection. Analysis of retrovirus-infected cells revealed Ago1 co-localized with siRNA in the nucleus, while Ago2 co-localized with siRNA in the inner nuclear envelope. Mismatched and scrambled siRNAs were observed in the cytoplasm, indicating sequence specificity. This is the first report directly visualizing nuclear compartment distribution of Ago-associated siRNA and further reveals a novel nuclear trafficking mechanism for RITS-like components involving the actin cytoskeleton. These results establish a model for elucidating mammalian TGS and suggest a fundamental mechanism underlying nuclear delivery of RITS-like components.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3287199/

1.2 Omissão da epigenética na concepção da transgenia

Estudos do genoma em organismos modelo e a condução do Projeto de Sequenciamento do Genoma Humano tornaram evidente que parte significativa das características físicas e químicas dos seres vivos (o fenótipo) não era apenas controlada pelo DNA e, consequentemente, pelos chamados genes. Há consenso, na atualidade, que várias dessas características são controladas por mecanismos epigenéticos, estabelecidos em patamares hierárquicos que extrapolam as funções atribuídas aos genes, cuja definição se mostra insuficiente.

A epigenética pode ser apresentada, de modo simplificado, como um meio de governança articulada, definidor da forma pela qual o genótipo poderá (ou não) ser utilizado para gerar a expressão de um ou outro fenótipo. Nesse sentido, nas palavras do biólogo Michel Morange, a epigenética "é um conceito que desmente em parte a 'fatalidade' dos genes".

Os mecanismos epigenéticos, que envolvem moléculas tais como RNA não codificadores/ncRNA (ou pequenos RNAs/sRNAs), são geralmente desencadeados/orientados por fatores ambientais, pela dieta ou ainda pelo microbioma (conjunto dos micro-organismos simbiontes dos seres vivos, tais como as bactérias da pele ou do sistema digestivo). Sob o conhecimento atual, tanto em plantas e artrópodes como em mamíferos[19] determinados mecanismos epigenéticos parecem ser universais, restando muito a descobrir sobre suas implicações. São enormes as dúvidas que permanecem relativamente ao funcionamento do epigenoma, seus diversos papéis biológicos nos organismos e os efeitos potencialmente indesejados que podem deflagrar desarranjos a eles impostos, pelo uso abusivo de tecnologias que o afetam (síntese de dsRNA transgênico/exógeno em especial).

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[19] Os mecanismos epigenéticos controlam funções biológicas chaves. O silenciamento permanente de um dos cromossomos X, nas fêmeas de mamíferos, destaca-se entre seus exemplos mais simbólicos.

COM – Bass BL. 2001. RNA interference: The short answer. *Nature*, 411: 428–9.

Sem resumo.

http://www.nature.com/nature/journal/v411/n6836/full/411428a0.html

Siomi, H.; Siomi, M. 2009. On the road to reading the RNA-interference code. *Nature*, 457: 396–404.

The finding that sequence-specific gene silencing occurs in response to the presence of double-stranded RNAs has had an enormous impact on biology, uncovering an unsuspected level of regulation of gene expression. This process, known as RNA interference (RNAi) or RNA silencing, involves small non-coding RNAs, which associate with nuclease-containing regulatory complexes and then pair with complementary messenger RNA targets, thereby preventing the expression of these mRNAs. Remarkable progress has been made towards understanding the underlying mechanisms of RNAi, raising the prospect of deciphering the 'RNAi code' that, like transcription factors, allows the fine-tuning and networking of complex suites of gene activity, thereby specifying cellular physiology and development.

http://www.ncbi.nlm.nih.gov/pubmed/19158785

Khan, A.; Betel, D.; Miller, M., Sander, C., Leslie, C., Marks, D. 2009. Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. *Nat. Biotechnol.*, 27(6):549-55. doi: 10.1038/nbt.1543. Erratum in *Nat. Biotechnol.*, 27(7):671.

Transfection of small RNAs (such as small interfering RNAs (siRNAs) and microRNAs (miRNAs)) into cells typically lowers expression of many genes. Unexpectedly, increased expression of genes also occurs. We investigated whether this upregulation results from a saturation effect--that is, competition among the transfected small RNAs and the endogenous pool of miRNAs for the intracellular machinery that processes small RNAs. To test this hypothesis, we analyzed genome-wide transcript responses from 151 published transfection experiments in seven different human cell types. We show that targets of endogenous miRNAs are expressed at significantly higher levels after transfection, consistent with impaired effectiveness of endogenous miRNA repression. This effect exhibited concentration and temporal dependence. Notably, the profile of endogenous miRNAs can be largely inferred by correlating miRNA sites with gene expression changes after transfections. The competition and saturation effects have practical implications for miRNA target prediction, the design of siRNA and short hairpin RNA (shRNA) genomic screens and siRNA therapeutics.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2782465/

Jagtap, U.; Gurav, R.; Bapat, V. 2011. Role of RNA interference in plant improvement. *Naturwissenschaften*, 98(6):473-92. doi: 10.1007/s00114-011-0798-8.

Research to alter crops for their better performance involving modern technology is underway in numerous plants, and achievements in transgenic plants are impacting crop improvements in unparalleled ways. Striking progress has been made using genetic engineering technology over the past two decades in manipulating genes from diverse and exotic sources, and inserting them into crop plants for inducing desirable characteristics. RNA interference (RNAi) has recently been identified as a natural mechanism for regulation of gene expression in all higher organisms from plants to humans and promises greater accuracy and precision to plant improvement. The expression of any gene can be down-regulated in a highly explicit manner exclusive of affecting the expression of any other gene by using RNAi technologies. Additional research in this field has been focused on a number of other areas including microRNAs, hairpin RNA, and promoter methylation. Manipulating new RNAi pathways, which generate small RNA molecules to amend gene expression in crops, can produce new quality traits and having better potentiality of protection against abiotic and biotic stresses. Nutritional improvement, change in morphology, or enhanced secondary metabolite synthesis are some of the other advantages of RNAi technology. In addition to its roles in regulating gene expression, RNAi is also used as a natural defense mechanism against molecular parasites such as jumping genes and viral genetic elements that affect genome stability. Even though much advancement has been made on the field of RNAi over the preceding few years, the full prospective of RNAi for crop improvement remains to be fully realized. The intricacy of RNAi pathway, the molecular machineries, and how it relates to plant development are still to be explained.

http://www.ncbi.nlm.nih.gov/pubmed/21503773

REV – Lejeune, E. ; Allshire, R. 2011. Common ground: small RNA programming and chromatin modifications. *Curr. Opin. Cell. Biol.*, 23:258–65.

Epigenetic mechanisms regulate genome structure and expression profiles in eukaryotes. RNA interference (RNAi) and other small RNA-based chromatin-modifying activities can act to reset the epigenetic landscape at defined chromatin domains. Centromeric heterochromatin assembly is a RNAi-dependent process in the fission yeast Schizosaccharomyces pombe, and provides a paradigm for detailed examination of such epigenetic processes. Here we review recent progress in understanding the mechanisms that underpin RNAi-mediated heterochromatin formation in S. pombe. We discuss recent analyses of the events that trigger RNAi and manipulations which uncouple RNAi and chromatin modification. Finally we provide an overview of similar molecular machineries across species where related small RNA pathways appear to drive the epigenetic reprogramming in germ cells and/or during early development in metazoans.

http://www.ncbi.nlm.nih.gov/pubmed/21478005

REV – Zhang H.; Zhu J-K. Seeing the forest for the trees: a wide perspective on RNA-directed DNA methylation. *Genes Dev.*, 26: 1769–73.

In this issue of Genes & Development, Wierzbicki and colleagues (pp. 1825-1836) examine the current model of RNA-directed DNA methylation (RdDM) by determining genome-wide distributions of RNA polymerase V (Pol V) occupancy, siRNAs, and DNA methylation. Their data support the key role of base-pairing between Pol V transcripts and siRNAs in targeting de novo DNA methylation. Importantly, the study also reveals unexpected complexity and provides a global view of the RdDM pathway.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3426756/

Cortessis, V.; Thomas D.; Levine A.; *et al*. 2012. Environmental epigenetics: prospects for studying epigenetic mediation of exposure–response relationships. *Hum Genet.,* 131: 1565–89.

Changes in epigenetic marks such as DNA methylation and histone acetylation are associated with a broad range of disease traits, including cancer, asthma, metabolic disorders, and various reproductive conditions. It seems plausible that changes in epigenetic state may be induced by environmental exposures such as malnutrition, tobacco smoke, air pollutants, metals, organic chemicals, other sources of oxidative stress, and the microbiome, particularly if the exposure occurs during key periods of development. Thus, epigenetic changes could represent an important pathway by which environmental factors influence disease risks, both within individuals and across generations. We discuss some of the challenges in studying epigenetic mediation of pathogenesis and describe some unique opportunities for exploring these phenomena.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3432200/

Shenderov, B.; Midtvedt, T. 2014. Epigenomic programing: a future way to health? *Microb. Ecol. Health Dis.,* 8;25. doi: 10.3402/mehd.v25.24145.

It is now generally accepted that the 'central genome dogma' (i.e. a causal chain going from DNA to RNA to proteins and downstream to biological functions) should be replaced by the 'fluid genome dogma', that is, complex feed-forward and feed-back cycles that interconnect organism and environment by epigenomic programing - and reprograming - throughout life and at all levels, sometimes also down the generations. The epigenomic programing is the net sum of interactions derived from own metabolism and microbiota as well as external factors such as diet, pharmaceuticals, environmental compounds, and so on. It is a growing body of results indicating that many chronic metabolic and degenerative disorders and diseases - often called 'civilization diseases' - are initiated and/or influenced upon by non-optimal epigenomic programing, often taking place early in life. In this context, the first 1,000 days of life - from conception into early infancy - is often called the most important period of life. The following sections present some major mechanisms for epigenomic programing as well as some factors assumed to be of importance. The need for more information about own genome and metagenome, as well as a substantial lack of adequate information regarding dietary and environmental databases are also commented upon. However, the mere fact that we can influence epigenomic health programing opens up the way for prophylactic and therapeutic interventions. The authors underline the importance of creating a 'Human Gut Microbiota and Epigenomic Platform' in order to facilitate interdisciplinary collaborations among scientists and clinicians engaged in host microbial ecology, nutrition, metagenomics, epigenomics and metabolomics as well as in disease epidemiology, prevention and treatment.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4016746/

Zhang, L.; Hou, D.; Chen, X.; *et al.* 2012. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Research* 22:107–126. doi:10.1038/cr.2011.158.

Our previous studies have demonstrated that stable microRNAs (miRNAs) in mammalian serum and plasma are actively secreted from tissues and cells and can serve as a novel class of biomarkers for diseases, and act as signaling molecules in intercellular communication. Here, we report the surprising finding that exogenous plant miRNAs are present in the sera and tissues of various animals and that these exogenous plant miRNAs are primarily acquired orally, through food intake. MIR168a is abundant in rice and is one of the most highly enriched exogenous plant miRNAs in the sera of Chinese subjects. Functional studies in vitro and in vivo demonstrated that MIR168a could bind to the human/mouse low-density lipoprotein receptor adapter protein 1 (LDLRAP1) mRNA, inhibit LDLRAP1 expression in liver, and consequently decrease LDL removal from mouse plasma. These findings demonstrate that exogenous plant miRNAs in food can regulate the expression of target genes in mammals.

Artigo completo disponível em http://www.nature.com/cr/journal/v22/n1/full/cr2011158a.html

Um excelente exemplo da precipitação da indústria em sua ânsia por colocar rapidamente no mercado produtos transgênicos ainda não bem compreendidos pela ciência diz respeito ao tomate Flav/Svr[20]. Primeira planta transgênica comercializada no mundo, foi distribuída ao consumo antes mesmo de serem conhecidos os mecanismos subjacentes aos efeitos obtidos pela manipulação genética. Apenas 12 anos após o lançamento daquele tomate no mercado, os pesquisadores alcançaram interpretações mais claras a respeito dos mecanismos biológicos responsáveis pelo amadurecimento controlado por um indutor químico, no momento desejado. Na ocasião do lançamento, supunha-se que o efeito obtido no tomate Flav/Svr decorria de mediação direta pela síntese de mRNA antissenso. Ora, hoje se reconhece que essa tecnologia recorria, na verdade, a um mecanismo epigenético de RNA interferência (RNAi).

Sanders, R.; Hiatt, W. 2005. Tomato transgene structure and silencing. *Nat. Biotechnol.,* 23: 287–9.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/15765076

Krieger, E.; Allen, E.; Gilbertson, L.; Roberts, J.; Hiatt, W.; Sanders, R. 2008. The Flavr Savr Tomato, an early example of RNAi technology. *HortScience*, 43, 962–964.

The Flavr Savr tomato was introduced as the first genetically engineered whole food in 1994. The commercial event, resulting from transformation with an antisense expression cassette of the endogenous *polygalacturonase* gene, was sequenced and found to contain two contiguous, linked, transfer DNA insertions. We found polygalacturonase suppression correlates with accumulation of ≈21-nt small interfering RNAs, the hallmark of an RNA interference-mediated suppression mechanism.

Artigo completo disponível em http://hortsci.ashspublications.org/content/43/3/962.full

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[20] Um erro de interpretação similar foi constatado no caso da papaia geneticamente modificada para resistir ao vírus da mancha anelar (Chiang et al., 2001. Comparative reactions of recombinant papaya ringspot viruses with chimeric coat protein (CP) genes and wild-type viruses on CP-transgenic papaya. *J Gen Virol* 82 (Pt 11): 2827–36).

2 Resposta do organismo geneticamente modificado à transgenia

2.1 A imprecisão da tecnologia de inserção do transgene resulta em modificações importantes no genoma do organismo transformado

Contrariamente ao que tem sido historicamente defendido pelas empresas de biotecnologias, o desenvolvimento de plantas transgênicas resulta de processo tecnológico altamente invasivo e aleatório, responsável por danos no DNA do organismo transformado e na configuração do próprio transgene (mutações, deleções, rearranjos, etc.).

Também têm sido observadas inserções em que os transgenes resultam fixados em locais instáveis do genoma, tais como em retrotransposons[21] (elementos móveis de DNA).

O número de cópias do cassete/transgene que serão integradas no genoma alvo também se mostra altamente variável e dependente do acaso. Deve-se ressaltar, neste ponto, que a cada cópia inserida corresponderá agregação de novas e adicionais incertezas quanto aos efeitos finais daquela modificação por transgenia.

> REV – Pawlowski, W.; Somers, D. 1996. Transgene inheritance in plants genetically engineered by microprojectile bombardment. *Mol. Biotechnol.*, 6 (1): 17-30.
>
> Microprojectile bombardment to deliver DNA into plant cells represents a major breakthrough in the development of plant transformation technologies and accordingly has resulted in transformation of numerous species considered recalcitrant to Agrobacterium- or protoplast-mediated transformation methods. This article attempts to review the current understanding of the molecular and genetic behavior of transgenes introduced by microprojectile bombardment. The characteristic features of the transgene integration pattern resulting from DNA delivery via microprojectile bombardment include integration of the full length transgene as well as rearranged copies of the introduced DNA. Copy number of both the transgene and rearranged fragments is often highly variable. Most frequently the multiple transgene copies and rearranged fragments are inherited as a single locus.

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[21] Numa visão evolutiva, é possível ilustrar a importância biológica dos retrotransposons em genoma de planta, com base no caso do milho. Para detalhes, ver SANMIGUEL, P. & BENNETZEN, J., L. 1998 (Evidence that a Recent Increase in Maize Genome Size was Caused by the Massive Amplification of Intergene Retrotransposons. *Annals of Botany* 82 (Supplement A): 37-44, 1998), disponível em http://aob.oxfordjournals.org/content/82/suppl_1/37.full.pdf.

However, a variable proportion of transgenic events produced by microprojectile bombardment exhibit Mendelian ratios for monogenic and digenic segregation vs events exhibiting segregation distortion. The potential mechanisms underlying these observations are discussed.

http://www.ncbi.nlm.nih.gov/pubmed/8887358

Srivastava V.; Anderson, O.; Ow, D. 1996. Single copy transgenic wheat generated through the resolution of complex integration patterns. *PNAS*, 96: 1117-21.

Genetic transformation of plants often results in multiple copies of the introduced DNA at a single locus. To ensure that only a single copy of a foreign gene resides in the plant genome, we used a strategy based on site-specific recombination. The transformation vector consists of a transgene flanked by recombination sites in an inverted orientation. Regardless of the number of copies integrated between the outermost transgenes, recombination between the outermost sites resolves the integrated molecules into a single copy. An example of this strategy has been demonstrated with wheat transformation, where four of four multiple-copy loci were resolved successfully into single-copy transgenes.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC17996/

Sawasaki, T.; Takahashi, M.; Goshima, N.; Morikawa, H. 1998. Structures of transgene loci in transgenic Arabidopsis plants obtained by particle bombardment: junction regions can bind to nuclear matrices. *Gene*, 218, 24-35.

To clarify the molecular structure of the integration sites of transgenes, we used particle bombardment to examine the DNA sequences of transgene loci. Three transgenic Arabidopsis lines gave a single Southern hybridization band with a selectable gene as the probe. Junction regions flanked by the transgenes were cloned by the inverse polymerase chain reaction method, and the characteristics of the DNA sequences of the 10 junction regions were investigated. All but two of these were AT-rich sequences bearing motifs characteristic of a scaffold/matrix-attachment region (S/MAR). Calculations showed that seven of them should have a propensity for curvature. An assay of in-vitro binding to tobacco nuclear matrices showed that all the junction regions bound to nuclear matrices and that the two input DNAs did not bind. The 12 chromosome/transgene (CT) junctions in these three transgene loci were investigated. Cleavage sites for topoisomerase I were found at 10 of the 12, near the junction point. The other two junctions had sites within 6bp of the junction point. The sequence near one terminal of the transgene in the transgene loci was compared with that near the other terminal. Short, direct repeats consisting of 4-6bp were present within 10bp of the junction points in the sequence. We speculate that the transgene introduced by particle bombardment is delivered on AT-rich S/MAR that has a propensity for curvature, and then a nucleotide near the short, direct repeat on the transgene is joined near the cleavage sites on the genome for topoisomerase I.

http://www.ncbi.nlm.nih.gov/pubmed/9751799

COM – Jank, B.; Haslberger, A. 2000. Recombinant DNA insertion into plant retrotransposons. *Tibtech,* Vol. 18.

Sem resumo.

Artigo completo disponível em http://www.ufv.br/DBV/PGFVG/BVE684/htms/pdfs_revisao/mutagenese_transposon/retrotransposonsplant.pdf

Svitashev, S.; Ananiev E.; Pawlowski, W.; Somers, D. 2000. Association of transgene integration sites with chromosome rearrangements in hexaploid oat. *Theor. Appl. Genet.* 100, 872-880.

Transgene loci in 16 transgenic oat (*Avena sativa* L.) lines produced by microprojectile bombardment were characterized using phenotypic and genotypic segregation, Southern blot analysis, and fluorescence in situ hybridization (FISH). Twenty-five transgene loci were detected; 8 lines exhibited single transgene loci and 8 lines had 2 or 3 loci. Double FISH of the transgene and oat C- and A/D-genome-specific dispersed and clustered repeats showed no preferences in the distribution of transgene loci among the highly heterochromatic C genome and the A/D genomes of hexaploid oat, nor among chromosomes within the genomes. Transgene integration sites were detected at different locations along individual chromosomes, although the majority of transformants had transgenes integrated into subtelomeric and telomeric regions. Transgene integration sites exhibited different levels of structural complexity, ranging from simple integration structures of two apparently contiguous transgene copies to tightly linked clusters of multiple copies of transgenes interspersed with oat DNA. The size of the genomic interspersions observed in these transgene clusters was estimated from FISH results on prometaphase chromosomes to be megabases long, indicating that some transgene loci were significantly larger than previously determined by Southern blot analysis. Overall, 6 of the 25 transgene loci were associated with rearranged chromosomes. These results suggest that particle bombardment-mediated transgene integration may result from and cause chromosomal breakage and rearrangements.

Artigo completo disponível em http://pawlowski.cit.cornell.edu/2000.pdf

Svitashevy S.; Pawlowskiy, W.; Makarevitch I.; Plank, D.; Somers D. 2002. Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement. *The Plant Journal*. 32, 433–445. **(1)**

To more fully characterize the internal structure of transgene loci and to gain further understanding of mechanisms of transgene locus formation, we sequenced more than 160 kb of complex transgene loci in two unrelated transgenic oat (Avena sativa L.) lines transformed using microprojectile bombardment. The transgene locus sequences from both lines exhibited extreme scrambling of non-contiguous transgene and genomic fragments recombined via illegitimate recombination. A perfect direct repeat of the delivered DNA, and inverted and imperfect direct repeats were detected in the same transgene locus indicating that homologous recombination and synthesis-dependent mechanism(s), respectively, were also involved in transgene locus rearrangement. The most unexpected result was the small size of the fragments of delivered and genomic DNA incorporated into the transgene loci via illegitimate recombination; 50 of the 82 delivered DNA fragments were shorter than 200 bp. Eleven transgene and genomic fragments were shorter than the DNA lengths required for Ku-mediated non-homologous end joining. Detection of these small fragments provided evidence that illegitimate recombination was most likely mediated by a synthesis-dependent strand-annealing mechanism that resulted in transgene scrambling. Taken together, these results indicate that transgene locus formation involves the concerted action of several DNA break-repair mechanisms.

http://www.ncbi.nlm.nih.gov/pubmed/12445116

Wu H.; Sparks, C.; Jones, H. 2006. Characterization of T-DNA loci and vector backbone sequences in transgenic wheat produced by Agrobacterium-mediated transformation. *Mol. Breeding* 18: 195-208.

Detailed molecular characterisation of transgene loci is a requirement for gaining regulatory approval for environmental release of genetically modified crops. In cereals, it is generally accepted that *Agrobacterium*-mediated transformation generates cleaner transgene loci with lower copy number and fewer rearrangements than those generated by biolistics. However, in wheat there has been little detailed analysis of T-DNA insertions at genetic and molecular level. Wheat lines transformed using*Agrobacterium tumefaciens* with *bar* and *gus*A (GUS) genes were subjected to genetic and molecular analysis. Unlike previous studies of transgene loci in wheat, we used functional assays for PAT and GUS proteins, combined with PCR and Southern analysis to detect the presence, copy number, linkage and transmission of two transgenes inserted in the same T-DNA. Thirty-four independent transgenic lines were categorised into three types: type I events (38% of total) where the *gus*A and *bar* genes displayed complete genetic linkage, segregating together as a single functional locus at the expected ratio of 3:1; type II events (18%), which possessed two or more transgene loci each containing *gus*A and *bar*; and type III events (44%), containing an incomplete T-DNA in which either the *gus*A or *bar* gene was lost. Most lines in this last category had lost the *bar*gene situated near the left T-DNA border. Southern analysis indicated that 30% of all lines possessed a single T-DNA copy containing *gus*A and *bar*. However, when data on expression and molecular analysis are combined, only 23% of all lines have single copy T-DNAs in which both gene cassettes are functioning. We also report on the presence of plasmid backbone DNA sequence in transgene loci detected using primer pairs outside the left and right T-DNA borders and within the plasmid selectable marker (*Npt*I) gene. Approximately two thirds of the lines contained some vector backbone DNA, more frequently adjacent to the left border. Taken together, these data imply unstable left border function causing premature T-strand termination or read-through into vector backbone. As far as we are aware, this is the first report revealing near border T-DNA truncation and vector backbone integration in wheat transgenic lines produced by *Agrobacterium*-mediated transformation.

http://link.springer.com/article/10.1007%2Fs11032-006-9027-0#close

REV – Wilson, A.; Latham, J.; Steinbrecher, R. 2006. Transformation-induced mutations in transgenic plants: analysis and biosafety implications. *Biotechnol. Genet. Eng.* 23:209-37.

Sem resumo.

Artigo completo disponível em http://www.econexus.info/publication/transformation-induced-mutations-transgenic-plants

REV – Latham, J.; Wilson, A.; Steinbrecher, R. 2006. The Mutational Consequences of Plant Transformation. *Journal of Biomedicine and Biotechnology*, Article ID 25376, Pages 1–7.

Plant transformation is a genetic engineering tool for introducing transgenes into plant genomes. It is now being used for the breeding of commercial crops. A central feature of transformation is insertion of the transgene into plant chromosomal DNA. Transgene insertion is infrequently, if ever, a precise event. Mutations found at transgene insertion sites include deletions and rearrangements of host chromosomal DNA and introduction of superfluous DNA. Insertion sites introduced using Agrobacterium tumefaciens tend to have simpler structures but can be associated with extensive chromosomal rearrangements, while those of particle bombardment appear invariably to be associated with deletion and extensive scrambling of inserted and chromosomal DNA. Ancillary procedures associated with plant transformation, including tissue culture and infection with A tumefaciens, can also introduce mutations. These genome-wide mutations can number from hundreds to many thousands per diploid genome. Despite the fact that confidence in the safety and dependability of crop species rests significantly on their genetic integrity, the frequency of transformation-induced mutations and their importance as potential biosafety hazards are poorly understood.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1559911/

Nesse contexto é necessário destacar o fato de que a maioria dos eventos transgênicos até hoje liberados comercialmente apresenta configurações de transgenes fisicamente distintos daqueles que estavam previstos e eram esperados como resultado do processo de inserção.

Windels, P.; Taverniers, I.; Depicker, A.; Van Bockstaele, E.; De Loose, M. 2001. Characterisation of the Roundup Ready soybean insert. *Eur. Food Res. Technol.,* 213, 107-112.

In this article we describe the isolation and characterisation of the junction between insert DNA and plant DNA in the transgenic Roundup Ready soybean line event 40-3-2. Our results establish that during integration of the insert DNA several rearrangements occurred at the 3' NOS junction and that the genomic plant DNA at the pre-integration site may have been rearranged. These findings highlight the utility of characterising junction regions to fulfil the request for information regarding which DNA sequences have been incorporated in commercialised transgenic lines. Furthermore, the characterisation of junction regions is, in our opinion, the method of choice to support method development for detection and identification of plant biotechnology-derived products.

Artigo completo disponível em http://cera-gmc.org/docs/articles/09-090-008.pdf

Hernández, M.; Pla, M.; Esteve, T.; Prat, S.; Puigdomènech, P.; Ferrando, A. 2003. A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard® based on the 3´-transgene integration sequence. *Transgenic Research,* 12: 179–189.

The increasing presence of transgenic plant derivatives in a wide range of animal and human consumables has provoked in western Europe a strong demand for appropriate detection methods to evaluate the existence of transgenic elements. Among the different techniques currently used, the real-time quantitative PCR is a powerful technology well adapted to the mandatory labeling requirements in the European Union (EU). The use of transgene flanking genomic sequences has recently been suggested as a means to avoid ambiguous results both in qualitative and quantitative PCR-based technologies. In this study we report the identification of genomic sequences adjacent to the 3'-integration site of event MON810 in transgenic maize. This genetically modified crop contains transgene sequences leading to ectopic expression of a synthetic CryIA(b) endotoxin which confers resistance to lepidopteran insects especially against the European corn borer. The characterization of the genome-transgene junction sequences by means of TAIL-PCR has facilitated the design of a specific, sensitive and accurate quantification method based on TaqMan chemistry. Cloning of event MON810 3'-junction region has also allowed to compare the suitability of plasmid target sequences versus genomic DNA obtained from certified reference materials (CRMs), to prepare standard calibration curves for quantification.

http://www.ncbi.nlm.nih.gov/pubmed/12739886

Rang, A.; Linke, B.; Jansen, B. 2005. Detection of RNA variants transcribed from the transgene in Roundup Ready soybean. *Eur. Food Res. Technol.,* 220, 438-43.

The acreage for genetically modified crops (GMOs)—particularly soybean—has steadily increased since 1996, when the first crop of Roundup Ready soybean (intended for food production) was grown. The Roundup Ready soybean varieties derive from a soybean line into which a glyphosate-resistant enolpyruvylshikimate- 3-phosphate-synthase (EPSPS) gene was introduced. The inserted and the flanking regions in Roundup Ready soybean have recently been characterized. It was shown that a further 250-bp fragment of the epsps gene is localized downstream of the introduced nos terminator of transcription, derived from the nopaline synthase gene from Agrobacterium tumefaciens. We examined whether this 250-bp fragment could be of functional importance. Our data demonstrate that at least 150 bp of this DNA region are transcribed in Roundup Ready soybean. Transcription of the fragment depends on whether readthrough events ignore the nos terminator signal located upstream. Our data also indicate that the read-through product is further processed, resulting in four different RNA variants from which the transcribed region of the nos terminator is completely deleted. Deletion results in the generation of open reading frames which might code for (as yet unknown) EPSPS fusion proteins. The nos terminator is used as a regulatory element in several other GMOs used for food production. This implies that read through products and transcription of RNA variants might be a common feature in these GMOs.

Artigo completo disponível em http://link.springer.com/article/10.1007%2Fs00217-004-1064-5#page-1

Collonnier, C.; Schattner, A.; Berthier, G.; *et al.* 2005. Characterization and event specific-detection by quantitative real-time PCR of T25 maize insert. *Journal AOAC International*, vol. 88, no. 2, p. 536-546.

T25 is one of the 4 maize transformation events from which commercial lines have so far been authorized in Europe. It was created by polyethylene glycol-mediated transformation using a construct bearing one copy of the synthetic pat gene associated with both promoter and terminator of the 35S ribosomal gene from cauliflower mosaic virus. In this article, we report the sequencing of the whole T25 insert and the characterization of its integration site by using a genome walking strategy. Our results confirmed that one intact copy of the initial construct had been integrated in the plant genome. They also revealed, at the 5' junction of the insert, the presence of a second truncated 35S promoter, probably resulting from rearrangements which may have occurred before or during integration of the plasmid DNA. The analysis of the junction fragments showed that the integration site of the insert presented high homologies with the Huck retrotransposon family. By using one primer annealing in the maize genome and the other in the 5' end of the integrated DNA, we developed a reliable event-specific detection system for T25 maize. To provide means to comply with the European regulation, a real-time PCR test was designed for specific quantitation of T25 event by using Taqman chemistry.

http://www.ncbi.nlm.nih.gov/pubmed/15859082

Rosati, A.; Bogani, P.; Santarlasci, A.; Bulatti, M. 2008. Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. *Plant Mol. Biol.*, 67 (3): 271-81.

The construct inserted in YieldGard MON810 maize, produced by Monsanto, contains the CaMV 35S promoter, the hsp70 intron of maize, the cryI(A)b gene for resistance to lepidopterans and the NOS terminator. In a previous work a truncation event at the 3' end of the cryI(A)b gene leading to the complete loss of the NOS terminator was demonstrated. The 3' maize genome junction region was isolated in the same experiment not showing any homology with known sequences. The aim of the experiments here reported was therefore to isolate and characterize a larger portion of the 3' integration junction from genomic DNA of two commercial MON810 maize lines. Specific primers were designed on the 3' integration junction sequence for the amplification of a 476 bp fragment downstream of the sequence previously detected. In silico analysis identified the whole isolated 3' genomic region as a gene putatively coding for the HECT E3 ubiquitin ligase. RT-PCR performed in this region produced cDNA variants of different length. In silico translation of these transcripts identified 2 and 18 putative additional aminoacids in different variants, all derived from the adjacent host genomic sequences, added to the truncated CRY1A protein. These putative recombinant proteins did not show homology with any known protein domains. Our data gave new insights on the genomic organization of MON810 in the YieldGard maize and confirmed the previous suggestion that the integration in the genome of maize caused a complex recombination event without, apparently, interfering with the activity of the partial CRY1A endotoxin and both the vigor and yield of the YieldGard maize.

Artigo completo disponível em http://www.salmone.org/wp-content/uploads/2009/05/buiatti2008.pdf

La Paz, J.; Vicient, C.; Puigdomènech, P.; Pla, M. 2010. Characterization of polyadenylated*cryIA(b)* transcripts in maize MON810 commercial varieties. *Analytical and Bioanalytical Chemistry*, Volume 396, Issue 6, pp 2125-2133.

The Zea mays L. event MON810 is one of the major commercialized genetically modified crops. The inserted expression cassette has a 3' truncation partially affecting the cryIA(b) coding sequence, resulting in the lack of the NOS terminator, with transcription of the transgene reported to read-through 3'-past the truncation site. Here, we demonstrate that the cryIA(b) transgene gives rise to a variety of polyadenylated transcripts of different sizes that extend to around 1 kbp downstream the truncation site. A Stop codon at position +7 downstream the truncation site indicates the production of a transgenic protein with two additional amino acids; which is compatible with the reported size of the CryIA(b) protein in MON810. There is no evidence of the existence of other translated products. Several main 3' transcription termination regions were detected close to the truncation site and in the transgene 3' flanking sequence. Next to these main termination sites, we identified some sequence motifs that could potentially act as 3'-end-processing elements and drive termination of the transgene transcripts. The MON810 transgene has been introduced into different commercial varieties through breeding programs. Here, we demonstrate that there are no significant differences among the levels of transgene mRNA accumulation, major transcript sizes and 3' termini profiles comparing a number of MON810 commercial varieties grown under similar environmental conditions. Commercial varieties of this event appear to be stable in terms of transgene expression.

http://www.ncbi.nlm.nih.gov/pubmed/19841912

Waminal, N.; Ryu, K.; Choi S-H.; Kim H. 2013. Randomly detected genetically modified (GM) maize *(Zea mays* L.) near a transport route revealed a fragile 45S rDNA phenotype. *PLOS ONE*. 9;8(9):e74060. doi: 10.1371/journal.pone.0074060.

Monitoring of genetically modified (GM) crops has been emphasized to prevent their potential effects on the environment and human health. Monitoring of the inadvertent dispersal of transgenic maize in several fields and transport routes in Korea was carried out by qualitative multiplex PCR, and molecular analyses were conducted to identify the events of the collected GM maize. Cytogenetic investigations through fluorescence in situ hybridization (FISH) of the GM maize were performed to check for possible changes in the 45S rDNA cluster because this cluster was reported to be sensitive to replication and transcription stress. Three GM maize kernels were collected from a transport route near Incheon port, Korea, and each was found to contain NK603, stacked MON863 x NK603, and stacked NK603 x MON810 inserts, respectively. Cytogenetic analysis of the GM maize containing the stacked NK603 x MON810 insert revealed two normal compact 5S rDNA signals, but the 45S rDNA showed a fragile phenotype, demonstrating a "beads-on-a-string" fragmentation pattern, which seems to be a consequence of genetic modification. Implications of the 45S rDNA cluster fragility in GM maize are also discussed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3767626/

Ben Ali, S-E.; Madi, Z.; Hochegger, R.; Quist, D.; Prewein, B.; Haslberger, A.; Brandes, C. 2014. Mutation Scanning in a Single and a Stacked Genetically Modified (GM) Event by Real-Time PCR and High Resolution Melting (HRM) Analysis. *Int. J. Mol. Sci., 15*, 19898-19923; doi:10.3390/ijms151119898.

Genetic mutations must be avoided during the production and use of seeds. In the European Union (EU), Directive 2001/18/EC requires any DNA construct introduced via transformation to be stable. Establishing genetic stability is critical for the approval of genetically modified organisms (GMOs). In this study, genetic stability of two GMOs was examined using high resolution melting (HRM) analysis and real-time polymerase chain reaction (PCR) employing Scorpion primers for amplification. The genetic variability of the transgenic insert and that of the flanking regions in a single oilseed rape variety (GT73) and a stacked maize (MON88017×MON810) was studied. The GT73 and the 5' region of MON810 showed no instabilities in the examined regions. However; two out of 100 analyzed samples carried a heterozygous point mutation in the 3' region of MON810 in the stacked variety. These results were verified by direct sequencing of the amplified PCR products as well as by sequencing of cloned PCR fragments. The occurrence of the mutation suggests that the 5' region is more suitable than the 3' region for the quantification of MON810. The identification of the single nucleotide polymorphism (SNP) in a stacked event is in contrast to the results of earlier studies of the same MON810 region in a single event where no DNA polymorphism was found.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264145/

Mesmo em casos em que a sequência de DNA mapeada na planta hospedeira se mostrasse idêntica àquela prevista, isso não asseguraria que suas operações viessem a reproduzir os efeitos esperados, e apenas estes. A semelhança em nível de identidade daquela informação genética não assegura por si só que a síntese de proteínas será idêntica no ser original e no ser transformado. De fato, mecanismos pós-traducionais – concretizados pelo dobramento no espaço tridimensional da sequência de aminoácidos – , dependentes do meio celular e das influências a que a célula se submete, definirão caminhos essenciais para configuração das funções biológicas definitivas daquela proteína. A adoção de uma conformação espacial distinta da esperada – independentemente do DNA que gerou determinada proteína – pode alterar sua funcionalidade, transformando macromoléculas inofensivas para o organismo em toxinas[22] ou alérgenos perigosos.

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[22] Cabe salientar que a diferença entre um tipo de príon normal e o príon responsável pela doença da vaca louca reside apenas nas conformações espaciais da proteína, e não da sequência de DNA que as geraram. Confira, por exemplo, Abid & Soto, 2006 (The intriguing prion disorders. *Cell Mol Life Sci,* 63, 2342–2351).

Prescott, V.; Campbell, P.; Moore, A.; Mattes, J.; Rothenberg, M.; Foster, P.; Higgins, T.; Hogan, S. 2005. Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. *Journal of Agricultural and Food Chemistry*, 53.

The development of modern gene technologies allows for the expression of recombinant proteins in non-native hosts. Diversity in translational and post-translational modification pathways between species could potentially lead to discrete changes in the molecular architecture of the expressed protein and subsequent cellular function and antigenicity. Here, we show that transgenic expression of a plant protein (alpha-amylase inhibitor-1 from the common bean (Phaseolus vulgaris L. cv. Tendergreen)) in a non-native host (transgenic pea (Pisum sativum L.)) led to the synthesis of a structurally modified form of this inhibitor. Employing models of inflammation, we demonstrated in mice that consumption of the modified alphaAI and not the native form predisposed to antigen-specific CD4+ Th2-type inflammation. Furthermore, consumption of the modified alphaAI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these proteins. Thus, transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants possessing altered immunogenicity.

http://www.ncbi.nlm.nih.gov/pubmed/16277398

2.2 Instabilidade do transgene – e da sua expressão – após inserção

A inserção forçada de material transgênico, que geralmente envolve elementos de DNA de várias espécies filogeneticamente distantes, tende a provocar alterações no genoma do organismo transformado, gerando condições favoráveis à instabilidade dos transgenes. Essa instabilidade – na expressão das proteínas recombinantes (ou em outros produtos de expressão) – pode se exprimir durante o ciclo vegetativo da planta e/ou no momento da transmissão do transgene de geração em geração.

Sendo vários os fatores internos e externos ao organismo que influenciam essas instabilidades, é evidente que seu conjunto gera riscos e incertezas bastante complexos de antecipação, em uma perspectiva de biossegurança.

Por vezes, o transgene é simplesmente silenciado[23], como já tem sido observado inclusive em variedades transgênicas comerciais.

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[23] O silenciamento gênico de transgenes é também tratado no item 2.3 da Parte 2 deste livro, onde discutimos ineficácia potencial de tecnologias de resistência a vírus em plantas geneticamente modificadas.

Meza, T.; Kamfjord, D.; Håkelien, A-M.; Evans, I.; Godager, L.; Mandal, A.; Jakobsen, K.; Aalen, R. 2001. The frequency of silencing in *Arabidopsis thaliana* varies highly between progeny of siblings and can be influenced by environmental factors. *Transgenic Research,* 10: 53–67.

In a collection of 111 transgenic Arabidopsis thaliana lines, silencing of the nptII gene was observed in 62 (56%) of the lines and three distinct nptII-silencing phenotypes were identified. Two T-DNA constructs were used, which differed in distance and orientation of the marker gene relative to the border sequences. Comparison of the sets of lines generated with each vector, indicate that the T-DNA construct configuration influence the incidence of lines displaying silencing, as well as the distribution of silencing phenotypes. Twenty lines were investigated more thoroughly. The frequency of silencing varied between siblings in 19 lines, including three lines containing a single T-DNA copy. The last line showed 100% silencing. The gus gene present in both constructs could be expressed in the presence of a silenced nptII gene. Investigation of methylation at a single site in the pnos promoter revealed partial methylation in multi-copy lines, but no methylation in single-copy lines. For 16 lines, the overall frequencies of silencing differed significantly between control plants and plants exposed to temperature stress; in 11 of these lines at the 0.1% level. In several cases, the frequency of silencing in progeny of stress-treated plants was higher than for the control group, while other lines showed higher frequencies of kanamycin-resistant progeny for the stress-treated sibling plants.

Artigo completo disponível em https://www.google.com.br/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=0CCcQFjAB&url=http%3A%2F%2Fwww.researchgate.net%2Fpublication%2F12080873_The_frequency_of_silencing_in_Arabidopsis_thaliana_varies_highly_between_progeny_of_siblings_and_can_be_influenced_by_environmental_factors%2Flinks%2F0912f505c734e5e7b9000000&ei=G0UtVOvmB7aLsQSBmoCwAw&usg=AFQjCNGaqMrzSRKGgIbuj1CVhhaPTXufIg&bvm=bv.76477589,d.cWc

Aguilera, M.; Querci, M.; Balla, B.; Prospero, A.; Ermolli, M.; Van den Eede, G. 2008. A Qualitative Approach for the Assessment of the Genetic Stability of the MON 810 Trait in Commercial Seed Maize Varieties. *Food Anal. Methods,* 1: 252-258.

Maize MON 810 is one of the European Union’s (EU) authorized genetically modified organisms (GMO) for placing on the food and feed market. The total number of MON 810 varieties registered in the European Common Catalogue of varieties of agricultural plant species has almost tripled since 2005. One of the requirements described in EU legislation, namely the genetic stability of GM seed varieties, was thus assessed by analyzing the intactness of the entire MON 810 integration and its genotypic stability in commercial varieties available on the market for at least the last 2 years. A combined strategy using qualitative analytical methods made possible to determine the presence/absence of the individual genetic elements and of the whole GM construct. The restriction fragment length polymorphism patterns obtained from amplified whole constructs by long polymerase chain reaction (PCR) were compared side by side. CryIA(b) protein expression levels were determined by enzyme-linked immunosorbent assay. Twenty-four out of the 26 analyzed varieties met the expected stability features. One variety gave negative results in all assays, and one variety contained the necessary genetic elements for expressing CryIA(b) protein although giving negative results for the long PCR product. To our knowledge, this study is the first post-marketing stability analysis performed on GM commercial seed varieties.

http://link.springer.com/article/10.1007%2Fs12161-008-9035-2

2.2.1 A instabilidade do transgene por fatores internos

Como mencionado anteriormente, além do método de transformação utilizado, também a estrutura organizacional decorrente da inserção do transgene influencia sua estabilidade, a estabilidade de sua expressão e, inclusive, o padrão de herança desses elementos de DNA ao longo das gerações.

Kohli, A.; Twyman, R.; Abranches, R.; Wegel, E.; Stoger, E.; Christou, P. 2003. Transgene integration, organization and interaction in plants. *Plant Molecular Biology* 52: 247–258.

It has been appreciated for many years that the structure of a transgene locus can have a major influence on the level and stability of transgene expression. Until recently, however, it has been common practice to discard plant lines with poor or unstable expression levels in favor of those with practical uses. In the last few years, an increasing number of experiments have been carried out with the primary aim of characterizing transgene loci and studying the fundamental links between locus structure and expression. Cereals have been at the forefront of this research because molecular, genetic and cytogenetic analysis can be carried out in parallel to examine transgene loci in detail. This review discusses what is known about the structure and organization of transgene loci in cereals, both at the molecular and cytogenetic levels. In the latter case, important links are beginning to be revealed between higher order locus organization, nuclear architecture, chromatin structure and transgene expression.

http://www.ncbi.nlm.nih.gov/pubmed/12856933

Yin, Z; Plader, W.; Malepszy, S. 2004. Transgene inheritance in plants. *J Appl Genet.*, 45(2):127-44.

The patterns of transgene inheritance in plants and the possible explanations for non-Mendelian transmission are reviewed. The non-Mendelian inheritance of a transgene has been recorded with a frequency between 10% and 50% in transgenic plants produced either by Agrobacterium-mediated transformation or through particle bombardment. Different effects such as deletion, duplication, rearrangement, repeated sequence recombination as well as gene interaction have been observed for transgenic loci. The nature of the recipient genome, nature of the transgene and the interactions between them seem to contribute to the non-Mendelian segregation of transgenes.

Artigo completo disponível em http://jag.igr.poznan.pl/2004-Volume-45/2/pdf/2004_Volume_45_2-127-144.pdf

Mecanismos epigenéticos também podem influenciar – e mesmo impedir – a expressão do transgene. Um dos caminhos se daria por meio do sistema denominado de PTGS (Post Transcriptional Gene Silencing). Esse modo de ação – que visa à destruição de todos os RNAm sintetizados por um transgene – é apontado como um tipo de "sistema de defesa genético" que reconhece e combate a introdução de determinadas sequências de DNA endógeno e/ou exógeno. De um ponto de vista evolucionista, esse mecanismo teria evoluído para proteger os organismos de vírus e transposons.

Outras informações sobre o mecanismo de PTGS envolvido na inibição da expressão transgênica no caso da tolerância a vírus podem ser encontradas no item 2.3 da Parte 2 deste livro.

Dougherty, W.; Parks, T.1995. Transgenes and gene suppression: telling us something new? *Curr Opin Cell Biol.,* Vol. 7(3): 399-405.

Transgenes provide unique opportunities to assess the relationship between genotype and phenotype in an organism. In most cases, introduction and subsequent expression of a transgene will increase (with a sense RNA) or decrease (with an antisense RNA) the steady-state level of a specific gene product. However, a number of surprising observations have been made in the course of many transgenic studies. We develop a hypothesis that suggests that many examples of endogenous gene suppression by either antisense or sense transcripts are mediated by the same cellular mechanism.

http://www.ncbi.nlm.nih.gov/pubmed/7662371

Kumpatla, S.; Chandrasekharan, M.; Iyer, M.; Li, G.; Hall, T. 1998. Genome intruder scanning and modulation systems and transgene silencing. *Trends in Plant Science*, Vol 3, Issue 3. 97-104.

The widespread occurrence of transgene inactivation in plants and classical cases of silencing of duplicated sequences in fungi suggest that all genomes contain defense systems that are capable of monitoring and manipulating intrusive DNA. Such DNA might be recognized by its structure, its sequence composition relative to that of its genomic environment and possibly by its disruption of normal biochemical functions. Although methylation, especially of repeated sequences, is widely associated with gene inactivation, other attributes, including chromatin modification, may be involved. Elimination of inactivated intrusive DNA (presently best documented for filamentous fungi) may also contribute to genomic defense mechanisms in plants. Stable integration and expression of introduced genes are essential for genetically engineered crops, and thus transformation constructs must be designed to avoid host surveillance processes.

Artigo completo disponível em http://healthcare.utah.edu/huntsmancancerinstitute/research/labs/chandrasekharan/images/pdfs/01_1998-Genome%20intruder%20scanning.pdf

Kumpatla, S.; Hall; T. 1999. Organizational complexity of a rice transgene locus susceptible to methylation-based silencing. *Life*, 48, 459-467.

Molecular analyses of a rice (Oryza sativa L.) transgene locus introduced using biolistic techniques revealed the presence of multiple copies of rearranged fragments, as well as an intact copy of the supplied constructs. Both the gene of interest (35S-Btt cryIIIA) and the selectable marker used (Ubi1-bar) were methylated and silenced. Additionally, vector sequences were present in great abundance and were also highly methylated, indicating that the entire transgene insert was marked for methylation. The rearrangement of input DNA resulted in interspersion of plasmid backbone regions with the gene of interest. Permutation of segments encoding the gene of interest and the selectable marker was also detected, perhaps explaining why sequences introduced on separate plasmids are frequently found to be inserted at the same locus. The 35S promoter contained several hotspots for fragmentation. These observations strongly support the concept that intrusive DNA is recognized by host surveillance systems and that transgene loci with anomalous structural organization are subjected to inactivation by processes such as methylation.

http://www.ncbi.nlm.nih.gov/pubmed/10632579

Cogoni, C.; Macino, G. 2000. Post-transcriptional gene silencing across kingdoms. *Curr Opinion Genet Dev*, 10, 638-43.

Post-transcriptional gene silencing (PTGS) as a consequence of the introduction of either transgenes or double-stranded RNA molecules has been found to occur in a number of species. In the past year, studies in different systems have greatly enhanced our understanding of the molecular mechanisms of these phenomena. The ubiquitous presence of PTGS in both the plant and animal kingdoms and the finding of common genetic mechanisms suggest that PTGS is a universal gene-regulation system fundamental in biological processes such as protection against viruses and transposons.

Artigo completo disponível em http://www.ufv.br/dbv/pgfvg/BVE684/htms/pdfs_revisao/expressaogenica/Post-transcriptional%20gene%20silencing%20across%20kingdoms.pdf

Hirai, S.; Takahashi, K.; Abiko, T.; Kodama, H. 2010. Loss of sense transgene-induced post-transcriptional gene silencing by sequential introduction of the same transgene sequences in tobacco. *The FEBS Journal*, 277(7):1695-703. doi: 10.1111/j.1742-4658.2010.07591.x.

RNA silencing is an epigenetic inhibition of gene expression and is guided by small interfering RNAs. Sense transgene-induced post-transcriptional gene silencing (S-PTGS) occurs in a portion of a transgenic plant population. When a sense transgene encoding a tobacco endoplasmic reticulum omega-3 fatty acid desaturase (NtFAD3) was introduced into tobacco plants, an S-PTGS line, S44, was obtained. Introduction of another copy of the NtFAD3 transgene into S44 plants caused a phenotypic change from S-PTGS to overexpression. Because this change was associated with the methylation of the promoter sequences of the transgene, reduced transcriptional activity may abolish S-PTGS and residual transcription of the sense transgene may account for the overexpression. To clarify whether RNA-directed DNA methylation (RdDM) can repress the transcriptional activity of the S44 transgene locus, we introduced several RdDM constructs targeting the transgene promoter. An RdDM construct harboring a 200-bp-long fragment of promoter sequences efficiently abrogated the generation of NtFAD3 small interfering RNAs in S44 plants. Transcription of the transgene was partially repressed, but the resulting NtFAD3 mRNAs successfully accumulated and an overexpressed phenotype was established. Our results indicate an example in which overexpression of the transgene is established by complex epigenetic interactions among the transgenic loci.

http://www.ncbi.nlm.nih.gov/pubmed/20180844

Cabe lembrar que situações em que a integração de um transgene ocorre fisicamente, mas sem este se expressar no organismo, já foram identificadas há mais de 30 anos. Apenas recentemente, entretanto, foram acessados conhecimentos que permitem interpretar alguns dos mecanismos envolvidos nesse processo.

Barton, K.; Binns, A.; Matzke, A. Chilton, M-D. 1983. Regeneration of Intact Tobacco Plants Containing Full Length Copies of Genetically Engineered T-DNA, and Transmission of T-DNA to RI Progeny. *Cell*, Vol. 32, 1033-l 043.

Cloned DNA sequences encoding yeast alcohol dehydrogenase and a bacterial neomycin phosphotransferase have been inserted into the T-DNA of Agrobacterium tumefaciens plasmid pTiT37 at the "rooty" locus. Transformation of tobacco stem segments with the engineered bacterial strains produced attenuated crown gall tumors that were capable of regeneration into intact, normal tobacco plants. The yeast gene and entire transferred DNA (T-DNA) were present in the regenerated plants in multiple copies, and nopaline was found in all tissues. The plants were fertile, and seedlings resulting from self-pollination also contained intact and multiple copies of the engineered T-DNA. Expression of nopaline in the germinated seedlings derived from one regenerated plant was variable and did not correlate with the levels of T-DNA present in the seedlings. Preliminary evidence indicates that nopaline in progeny of other similarly engineered plants is more uniform. The disarming of pTiT37 by insertions at the "rooty" locus thus appears to produce a useful gene vector for higher plants.

Artigo completo disponível em http://perso.univ-rennes1.fr/antoine.gravot/index.htm/M1%20BiotecVeg%202011-2012/BIBLIO%20COURS/1983-Barton%20RegenerationofTobaccoPlantsWithGeneticallyEngineeredTDNA%20CELL.pdf

2.2.2 A instabilidade do transgene por interações com fatores ambientais

Além dos fatores internos, existem inúmeros fatores ambientais externos que interagem com o transgene, afetando sua estabilidade. Em muitas circunstâncias, as condições ambientais locais e os fatores de estresse, a campo, parecem afetar a forma e as características de expressão dos transgenes.

Olsen, K.; Daly, J.; Finnegan, E.; Mahon, R. 2005. Changes in Cry1Ac Bt Transgenic Cotton in Response to Two Environmental Factors: Temperature and Insect Damage. *J. Econ. Entomol.* 98(4): 1382Ð1390.

The efficacy of Cry1Ac Bacillus thuringiensis (Bt) cotton plants against field populations of Helicoverpa armigera (Hübner) has been inconsistent over the growing season. Any reduction in efficacy (where efficacy is the capacity of the plant to affect the survival of the insect) increases the opportunities for H. armigera to evolve resistance to Bt toxin. Changes in efficacy could be due to changes at the level of gene expression and/or in the physiological makeup of the plant and may be induced by environmental conditions. Two environmental factors, temperature and insect damage, were investigated. Temperature was found to affect efficacy, whether plants were grown at different temperatures continuously or were exposed to a change in temperature for a short period. Damage caused by chewing insects (H. armigera larvae) produced a dramatic increase in the efficacy of presquare Bt cotton. In contrast, damage by sucking insects (aphids) did not induce changes in efficacy. Changes in efficacy seemed to be mediated through modification of the physiological background of the plant rather than changes in the level of Cry1Ac expression or in the concentration of the Bt toxin. The impact of the non-Bt responses of plants on strains of H. armigera should be evaluated. It is possible that by enhancing existing defensive mechanisms of plants, the rate of evolution of resistance to Bt toxins could be retarded by increasing the plants overall toxicity through the additive effects of the toxins and plant defenses.

http://www.ncbi.nlm.nih.gov/pubmed/16156594

Bruns, H.; Abel, C. 2007. Effects of nitrogen fertility on Bt endotoxin levels in maize. *Journal of Entomological Science*, 42: 35-43.

A study was conducted to determine if Bt endotoxin concentrations during reproductive growth of Bt maize hybrids are affected by different N-fertility rates used to grow the crop. Previous research has shown N-fertility rates positively affect Bt concentrations of young Bt maize plants grown in a glasshouse. Three Bt hybrids, two with Bt event MON-810 (AgriGold brand cv. A 6729Bt and Pioneer brand cv. 33V08Bt) one with Bt event DBT 418 (DeKalb 626Bt) and one non-Bt maize hybrid (DeKalb brand cv. 626) were grown at Stoneville, MS, USA in 2002 and 2003 with N-fertility rates of 0, 112, 224, and 336 kg N/ha. Tissue samples of the outer ear husks and primary ear leaf sheaths were collect at growth stage R3 and analyzed for Bt concentration. Agronomic data were collected at maturity. The concentrations of Bt endotoxin in both sets of tissue were positively correlated with N-fertility rate for the MON-810 Bt hybrids but not the DBT 418 Bt hybrid. Increases in N-fertility increased grain yields. The Bt hybrids had less lodging (0.7%-1.0%) than the non-Bt hybrid (5.1%). Adequate levels of N-fertility are important to MON-810 Bt hybrids not only for yield, but also to ensure sufficient levels of Bt endotoxin for maximum protection from susceptible insect pests.

Artigo completo disponível em http://www.regional.org.au/au/asa/2004/poster/3/8/453_brunsha.htm

Griffiths, B.; Caul, S.; Thompson, J.; Birch, A.; Scrimgeour, C.; Cortet, J.; Foggo, A.; Hacket, C.; Krogh, P.; 2006. Soil microbial and faunal community responses to Bt maize and insecticide in two soils. *Journal of Environmental Quality*, 35: 734-741.

The effects of maize (Zea mays L.), genetically modified to express the Cry1Ab protein (Bt), and an insecticide on soil microbial and faunal communities were assessed in a glasshouse experiment. Soil for the experiment was taken from field sites where the same maize cultivars were grown to allow comparison between results under glasshouse conditions with those from field trials. Plants were grown in contrasting sandy loam and clay loam soils, half were sprayed with a pyrethroid insecticide (deltamethrin) and soil samples taken at the five-leaf stage, flowering, and maturity. The main effect on all measured parameters was that of soil type and there were no effects of Bt trait or insecticide on plant growth. The Bt trait resulted in more soil nematodes and protozoa (amoebae), whereas insecticide application increased plant Bt concentration and altered nematode community structure. The only significant effects on soil microbial community structure, microarthropods, and larvae of a nontarget root-feeding Dipteran, were due to soil type and plant growth stage. The results indicate that, although there were statistically significant effects of the Bt trait on soil populations, they were small. The relative magnitude of the effect could best be judged by comparison with the insecticide treatment, which was representative of current best practice. The Bt trait had no greater effect than the insecticide treatment. Results from this glasshouse experiment were in broad agreement with conclusions from field experiments using the same plant material grown in the same soils.

Artigo completo disponível em http://www.dzumenvis.nic.in/GM%20Crops/pdf/Soil%20Microbial%20and%20Faunal%20Community.pdf

Zeller, S.; Kalinina, O.; Brunner, S.; Keller, B.; Schmid, B. 2010. Transgene X environment interactions in genetically modified wheat. *PLOS One*, 12; 5(7):e11405.

Background: The introduction of transgenes into plants may cause unintended phenotypic effects which could have an impact on the plant itself and the environment. Little is published in the scientific literature about the interrelation of environmental factors and possible unintended effects in genetically modified (GM) plants.
Methods and Findings: We studied transgenic bread wheat *Triticum aestivum* lines expressing the wheat *Pm3b* gene against the fungus powdery mildew *Blumeria graminis* f.sp. *tritici*. Four

independent offspring pairs, each consisting of a GM line and its corresponding non-GM control line, were grown under different soil nutrient conditions and with and without fungicide treatment in the glasshouse. Furthermore, we performed a field experiment with a similar design to validate our glasshouse results. The transgene increased the resistance to powdery mildew in all environments. However, GM plants reacted sensitive to fungicide spraying in the glasshouse. Without fungicide treatment, in the glasshouse GM lines had increased vegetative biomass and seed number and a twofold yield compared with control lines. In the field these results were reversed. Fertilization generally increased GM/control differences in the glasshouse but not in the field. Two of four GM lines showed up to 56% yield reduction and a 40-fold increase of infection with ergot disease *Claviceps purpurea* compared with their control lines in the field experiment; one GM line was very similar to its control.
Conclusions: Our results demonstrate that, depending on the insertion event, a particular transgene can have large effects on the entire phenotype of a plant and that these effects can sometimes be reversed when plants are moved from the glasshouse to the field. However, it remains unclear which mechanisms underlie these effects and how they may affect concepts in molecular plant breeding and plant evolutionary ecology.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0011405

Blaise, D.; Kranthi, K. 2011. Cry1Ac expression in transgenic Bt cotton hybrids is influenced by soil moisture and depth. *Current Science*, Vol 101 (6).

Cry1Ac toxin concentration was assessed in leaves of *Bt* transgenic cotton hybrid grown on shallow (<60 cm) and deep (>90 cm) black soils of Nagpur, Maharashtra, India. Cry toxin concentration increased up to 80 days after sowing followed by a steep decline. In general, toxin concentration was greater on the deep black soils than the shallow soil. This was because of greater water-holding capacity of the deep soils. Cry toxin concentration was closely related to the soil water content. Beyond (excess moisture) and below (moisture deficit) field capacity, toxin concentration declined. A cubic polynomial best described the relationship between Cry toxin concentration and soil moisture content ($R2 = 0.95$).

Artigo completo disponível em http://www.currentscience.ac.in/Volumes/101/06/0783.pdf

No caso das plantas Bt, a instabilidade na síntese das toxinas (de um ponto de vista quantitativo) prejudica a estratégia de controle de pragas com base em altas doses, utilizada em eventos mais recentes com vistas a limitar o desenvolvimento de populações de insetos geneticamente resistentes. Evidentemente essa instabilidade também abre campo para outras implicações e riscos, como perspectivas agronômicas, socioeconômicas e ambientais.

Coviella, C.; Morgan, D.; Trumble, J. 2000. Interactions of Elevated CO2 and Nitrogen Fertilization: Effects on Production of Bacillus thuringiensis Toxins in Transgenic Plants. *Environ. Entomol.*, 29(4): 781-787.

Elevated atmospheric CO_2 concentrations will cause plants to grow faster, lower nitrogen content per unit of plant tissue, and generate higher carbon to nitrogen (C/N) ratios. We hypothesize that production of transgenic proteins will be reduced, thus reducing the efficiency of *Bacillus thuringiensis* (Bt) transgenes against insect populations. Commercially available transgenic cotton plants expressing the *Cry 1Ac* gene from Bt were compared with a near isogenic non-Bt cotton line in a split-plot design with two levels of atmospheric CO_2 (ambient, 370 ppm and elevated, 900 ppm) incorporating a 2 × 2 factorial design with two nitrogen (N) fertilization regimes (low, 30 mg N/kg soil/wk and high, 130 mg N/kg soil/wk), and two levels of Bt (presence or absence). Bioassays using *Spodoptera exigua* (Hübner) and quantitative enzyme-linked immunosorbent assays for toxin content indicated reduced Bt protein production in elevated CO_2. The tendency for test insects to consume more foliage from plants with lower N, caused by the elevated CO_2, did not compensate for the reduction in toxin production. N fertilization regime interacted with CO_2 concentration, showing that plants growing in N limited systems would produce substantially less toxin. The use of transgenic plants is becoming increasingly important and will continue to be so in the next decades. At the same time, atmospheric CO_2 increase will affect the effectiveness of this strategy. These observations have implications not only for agricultural use of transgenic plants, but also for the ecological consequences of transfer of Bt toxins to closely related wild plant genotypes.

http://www.bioone.org/doi/abs/10.1603/0046-225X-29.4.781?journalCode=enve

Abel, C.; Adamczyk, J. 2004. Relative concentration of Cry1A in maize leaves and cotton bolls with diverse chlorophyll content and corresponding larval development of fall armyworm (*Lepidoptera: Noctuldae*) and southwestern corn borer (*Lepidoptera: Crambidae*) on maize whorle leaf profiles. *Journal of Economic Entomology,* 97: 1737-1744.

To manage insect resistance to transgenic crops that express insecticidal proteins from Bacillus thuringiensis (Bt) Berliner, the U.S. Environmental Protection Agency recommends a refuge-based insect resistance management strategy where a percentage of non-Bt (refuge) crop is grown in proximity to a Bt-expressing crop. An important requirement for this strategy is that the toxin exists at a high effective dose for control of the target pest(s), so that heterozygous individuals in the population do not reach adulthood. Factors that cause reduced levels of toxin in the plant are a threat to this strategy. We quantified Cry1Ab from different areas of the maize, Zea mays L., leaf. In general, the distal tip of the V7 maize leaf had a higher concentration of Cry1Ab compared with the middle section of the V7 leaf, and the middle section of the developing V9 leaf had the lowest concentration of Cry1Ab. When these sections of maize tissue were fed to fall armyworm, Spodoptera frugiperda (J.E. Smith), and southwestern corn borer, Diatraea grandiosella Dyar, there was not a reduction in development or an increase in mortality with tissue that had higher concentrations of toxin. Another study tested the relative concentration of Cry1Ab between the white-yellow, yellow-green, and green portions of the developing ninth leaf within the maize whorl. There were differences in Cry1Ab concentration among these leaf areas. The green tissue had the highest concentration of toxin followed by the yellow-green and white-yellow tissues. Correlations between concentration of Cry1Ab and 5-d fall armyworm larval weights among the three leaf color profiles were all significant and negative, i.e., decreased concentration of Cry1Ab in the leaf tissue resulted in increased 5-d larval weights. There was 100% mortality to the southwestern corn borer larvae fed Cry1Ab maize leaf tissue. Differences in the amount of Cry1Ab in the developing V9 leaf profiles did not alter the absolute susceptibility of the southwestern corn borer to the toxin. In cotton, Gossypium hirsutum L., the amount of Cry1Ac was significantly lower in boll tips where flowers had remained attached compared with normal boll tips. Boll tips where the flowers remained attached are often the site where corn earworms, Helicoverpa zea (Boddie), penetrate Bt cotton bolls. This study demonstrated that, in two diverse plant species, tissue that has low chlorophyll content does not fully express Cry1A. Photosynthesis regulating factors related to mRNA transcription and translation should be studied for their effect on Cry1A production and insect control.

http://www.ncbi.nlm.nih.gov/pubmed/15568367

Kranthi, K.; Naidu, S.; Dhawad, C; Tatwawadi, A.; Mate, K.; Patil, E.; Bharose, A.; Behere, G.; Wadaskar, R.; Kranthi, S. 2005. Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (*Noctuidae: Lepidoptera*). *Current Science*, Vol. 89 (2), 291.

The quantitative levels of Cry1Ac and the seasonal decline in expression differed significantly among the eight commercial Bollgard hybrids tested. The Cry1Ac expression was found to be variable among the hybrids and also between different plant parts. The leaves of *Bt*-cotton plants were found to have the highest levels of Cry1Ac expression followed by squares, bolls and flowers. The toxin expression in the boll-rind, square bud and ovary of flowers was clearly inadequate to confer full protection to the fruiting parts. Increasing levels of *Helicoverpa armigera* survival were correlated with the toxin levels decreasing below 1.8 mg/g in the plant parts. Genotype-independent seasonal decline of the Cry1Ac toxin levels was observed in all the hybrids. Cry1Ac expression decreased consistently as the plant aged. The decline in Cry1Ac was more rapid in some hybrids compared to others. The choice of parental background appeared to be crucial for sustainable expression of the *cry1Ac* transgene. The implications of variability in Cry1Ac expression and the seasonal decline on bollworm management are discussed.

Artigo completo disponível em http://www.greenpeace.org/india/Global/india/report/2005/8/exerpt-from-current-science.pdf

Dong, H.; Li, W. 2007. Variability of endotoxin expression in Bt transgenic cotton. *J. Agron. Crop Sci.,* 193 (1), 21-29.

Transgenic cotton expressing Bt (Bacillus thuringiensis) *toxins* is currently cultivated on a large commercial scale in many *countries*, but observations have shown that it behaves variably in toxin efficacy against target insects under field conditions. Understanding of the temporal and spatial variation in efficacy and the resulting mechanisms is essential for cotton protection and production. In this review, we summarize current knowledge on variability in Bt cotton efficacy, in particular on the induced variability by environmental stresses. We also discuss the resulting mechanisms and the countermeasures for the inconsistence in efficacy in Bt cotton. It is indicated that insecticidal *protein* content in Bt cotton is variable with plant age, plant structure or under certain environmental stresses. Variability in Bt cotton efficacy against target insect pests is mainly attributed to the changes in Bt protein content, but physiological changes associated with the production of secondary compounds in plant tissues may also play an important role. Reduction of Bt protein content in late-season cotton could be due to the overexpression of Bt gene at earlier stages, which leads to gene regulation at post-transcription *levels* and consequently results in gene silencing at a later stage. Methylation of the promotor may be also involved in the declined expression of endotoxin *proteins*. As a part of total protein, the insecticidal protein in plant tissues changes its level through inhibited synthesis, degradation or translocation to developing plant parts, particularly under environmental stresses, thus being closely correlated to N metabolism. It can be concluded that developing new cotton varieties with more powerful resistance, applying certain plant growth regulators, enhancing intra-plant defensive capability, and maintenance of general health of the transgenic crop are important in realizing the full transgenic potential in Bt cotton.

http://onlinelibrary.wiley.com/doi/10.1111/j.1439-037X.2006.00240.x/abstract

Nguyen, H.; Jehle, J.; 2007. Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. *Journal of Plant Diseases and Protection*, 114 (2), 82–87.

The tissue-specific expression and seasonal abundance of Cry1Ab *protein* were determined in transgenic maize plants (Mon810, variety 'Novelis') from two field trials located near Bonn and

Halle, Germany. *A total* of 1085 *samples* were analysed by using Double Antiserum-Enzyme Linked Immunosorbent Assay (DAS-ELISA). The Cry1Ab contents of various plant tissues (root, stem, upper leaf, lower leaf, anther, pollen and kernel) were determined at four different growth stages (BBCH19, BBCH30, BBCH61 and BBCH83) collected in 2001, 2002 and 2003. Mon810 showed the highest Cry1Ab contents in the leaves (5.5 - 6.4 mu g g(-1) fresh weight [fw]) at BBCH83, whereas the lowest Cry1Ab contents were detected in the pollen (1 - 97 ng g(-1) fw). Cry1Ab *content of* residual root stocks collected in the field nine months after harvest was 15 - 17 ng g(-1) fw. This demonstrated that the Cry1Ab concentration in residual root stocks was reduced to about one-hundredth of the fresh roots. The monitoring of Cry1Ab expression showed that the Cry1Ab contents varied strongly between different plant individuals.

Artigo completo disponível em http://www.jpdp-online.com/Artikel.dll/nguyen_MjQ5ODEz.PDF?UID=8DB00985B0C39C4CCB831B999FB51F768BE9A64EFAAC23B7

Bakhsh, A.; Shahzad, K.; Husnain, T. 2011. Variation in the spatio-temporal expression of insecticidal genes in cotton. *Czech J. Genet. Plant Breed.*, 47 (2011): 1-9

The most significant breakthrough in plant biotechnology is the development of the techniques to transform genes from unrelated sources into commercially important crop plants to develop resistance against targeted insect pests. The spatio-temporal expression of insecticidal genes in transgenic cotton varies with plant age, plant parts and environmental conditions. The understanding of this temporal and spatial variation in efficacy and the resulting mechanisms is essential for cotton protection and production. This review summarizes variations in the efficacy of introduced insecticidal genes in cotton crop. The factors contributing to the variability of endotoxins have also been highlighted. The reduction in *Bt* protein biosynthesis in late-season cotton tissues could be attributed to the overexpression of the Bt gene at earlier stages, which leads to gene regulation at post-transcription levels and consequently results in gene silencing at a later stage. Methylation of the promoter may also play a role in the declined expression of endotoxin proteins. In genetically modified crops several environmental factors have been reported to affect the expression of transgenes. Among environmental factors nitrogen metabolism, inhibition of synthesis, degradation, remobilization and high temperature are attributable to the quantitative reduction in Bt proteins. Applying plant growth regulators or protein enhancers such as Chaperone™ may improve Bt cotton efficacy through enhancing the synthesis of proteins. Also some agronomic practices such as nitrogen fertilization and timely irrigation favour the endotoxin expression. Thus, variations in the efficacy of insecticidal genes in transgenic cotton and the involved mechanisms need to be understood fully so as to plan rational resistance management strategies to retard the rate of resistance development and to control target pests effectively by enhancing the endotoxin expression through genetic or agronomic management.

Artigo completo disponível em http://www.agriculturejournals.cz/publicFiles/36993.pdf

Mejia, R.; Polanía, I.; 2012. Expression of the Cry1Ab Toxin in Transgenic Corn Yieldgard® in the Eastern Plains of Colombia. *Southwestern Entomologist,* 37(2):209-223.

The knowledge of the Bt toxin concentracion within a transgenic plant is basic for the estimation of the performance of the insect pest aimed to be managed with this technology. In a *commercial* lot at the National Cereal Federation (FENALCE) in the municipality of Granada (Meta), *Colombia*, *samples* from plants of transgenic maize YieldGard® were taken to determine the average concentracion of the Cry1Ab toxin. In the laboratory by means of the sandwich ELISA assay, the*protein* was quantified in vegetative and reproductive tissues with the kit "for Cry1Ab/Cry1Ac Qualiplate Envirologix." For this purpose, leaves, stem, ear, ear styles, husk, cob, and corn kernels were lyophilized. The analyses indicated that expression fluctuates between tissues from the same plant and between plants in the same plot. The average concentracion of

Cry1Ab was: 8.97 µg/g fresh weight in leaves (V7), 8.96 in stems, 2.3 in male inflorescence (VT), 9.57 in unfertilized styles, 7.27 in fertilized styles (R1), 8.39 in cob (R3), 12.19 in husks, and 1.85 µg/g fresh weight in grains (R3). concentracions in seeds and leaves were similar to those obtained in the U.S.A. and Europe. This marked difference in the toxin expression makes Bt corn a plant susceptible to some pests, because there is no uniformity in the Cry1Ab concentracion in the tissues consumed.

http://www.bioone.org/doi/abs/10.3958/059.037.0214

2.3 Diversidade de efeitos inesperados na expressão gênica, no metaboloma ou no proteoma do organismo transformado

A inserção/transferência de determinada sequência genética em outro organismo pode induzir/ativar novos e inesperados efeitos sobre a expressão de características fisiológicas/biológicas desse organismo transformado. Tais mudanças podem ser associadas ou não às alterações físicas resultantes do processo de transgenia ou mesmo a instabilidades do transgene, conforme mencionado nos itens precedentes (2.1 e 2.2).

Alguns desses efeitos podem ser observados por meio de avaliação do conjunto de proteínas e/ou metabólitos sintetizados pelo organismo transformado. A teoria que fundamenta a transgenia – baseada na hipótese fraca de que a transferência de apenas um "gene" resultaria exclusivamente na sua expressão íntegra e similar ao seu organismo de origem – implicaria que os produtos de expressão do transgene representariam as únicas diferenças entre o OGM e seu isogênico (organismo com genoma idêntico à exceção do transgene). Como consequência, a identificação de diferenças metabólicas distintas das previstas, após as inserções, deveria ser suficiente para caracterizar a fragilidade daquela hipótese e das interpretações dali decorrentes.

Ora, vários estudos constatam situações em que podem ser mapeadas dezenas de proteínas cujas expressões se fizeram modificadas pela inserção de um transgene. O fato é tão relevante que, a partir do conhecimento e de estudos do Metaboloma, evidenciando a possibilidade de alteração do perfil metabólico dos OGMs, a Comunidade Econômica Europeia passou a obrigar (desde 2004) a rotulagem – como "transgênicos" – de alimentos contendo mais de 0,9% de componente oriundo de ingredientes extraídos de OGMs[24].

Villas-Boas, S.; Gombert, A. 2006. Análise do Metaboloma. *Biotecnologia, Ciência e Desenvolvimento,* nº 36 p.58-69.

Sem resumo.

Artigo completo disponível em http://www.biotecnologia.com.br/revista/bio36/metaboloma_36.pdf

Batista, R.; Saibo, N.; Lourenço, T.; Oliveira, M. 2008. Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion. 3640–3645, PNAS, vol. 105, nº. 9.

Controversy regarding genetically modified (GM) plants and their potential impact on human health contrasts with the tacit acceptance of other plants that were also modified, but not considered as GM products (e.g., varieties raised through conventional breeding such as mutagenesis). What is beyond the phenotype of these improved plants? Should mutagenized plants be treated differently from transgenics? We have evaluated the extent of transcriptome modification occurring during rice improvement through transgenesis versus mutation breeding. We used oligonucleotide microarrays to analyze gene expression in four different pools of four types of rice plants and respective controls: (*i*) a γ-irradiated stable mutant, (*ii*) the M1 generation of a 100-Gy γ-irradiated plant, (*iii*) a stable transgenic plant obtained for production of an anticancer antibody, and (*iv*) the T1 generation of a transgenic plant produced aiming for abiotic stress improvement, and all of the unmodified original genotypes as controls. We found that the improvement of a plant variety through the acquisition of a new desired trait, using either mutagenesis or transgenesis, may cause stress and thus lead to an altered expression of untargeted genes. In all of the cases studied, the observed alteration was more extensive in mutagenized than in transgenic plants. We propose that the safety assessment of improved plant varieties should be carried out on a case-by-case basis and not simply restricted to foods obtained through genetic engineering.

Artigo completo disponível em http://www.pnas.org/content/105/9/3640.full

Liu, Z.; Li, Y.; Zhao, J.; Chen, X.; Jian, G.; Peng, Y.; Qi , F. 2012. Differentially Expressed Genes Distributed Over Chromosomes and Implicated in Certain Biological Processes for Site Insertion Genetically Modified Rice Kemingdao. *Int J Biol Sci.*, 8(7): 953-963.

Release of genetically modified (GM) plants has sparked off intensive debates worldwide partly because of concerns about potential adverse unintended effects of GM plants to the agro system and the safety of foods. In this study, with the aim of revealing the molecular basis for unintended effects of a single site insertion GM Kemingdao (KMD) rice transformed with a synthetic cry1Ab

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[24] No Brasil, o Decreto nº 4.680, de 24 de abril de 2003, que regulamenta a rotulagem de produtos que contém OGM, é também baseado em parte nessa fundamentação.

gene, and bridging unintended effects of KMD rice through clues of differentially expressed genes, comparative transcriptome analyses were performed for GM KMD rice and its parent rice of Xiushui11 (XS11). The results showed that 680 differentially expressed transcripts were identified from 30-day old seedlings of GM KMD rice. The absolute majority of these changed expression transcripts dispersed and located over all rice chromosomes, and existed physical distance on chromosome from the insertion site, while only two transcripts were found to be differentially expressed within the 21 genes located within 100 kb up and down-stream of the insertion site. Pathway and biology function analyses further revealed that differentially expressed transcripts of KMD rice were involved in certain biological processes, and mainly implicated in two types of pathways. One type was pathways implicated in plant stress/defense responses, which were considerably in coordination with the reported unintended effects of KMD rice, which were more susceptible to rice diseases compared to its parent rice XS11; the other type was pathways associated with amino acids metabolism. With this clue, new unintended effects for changes in amino acids synthesis of KMD rice leaves were successfully revealed. Such that an actual case was firstly provided for identification of unintended effects in GM plants by comparative transciptome analysis.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3399318/

Barbosa, H., S., Arruda, S., Azevedo, R., Arruda, M., 2012. New insights on proteomics of transgenic soybean seeds: evaluation of differential expressions of enzymes and proteins. *Analytical and Bioanalytical Chemistry*, January 2012, Volume 402, Issue 1, pp 299-314.

This work reports the evaluation of differentially expressed enzymes and proteins from transgenic and nontransgenic soybean seeds. Analysis of malondialdehyde, ascorbate peroxidase (EC 1.11.1.11), glutathione reductase (EC 1.6.4.2), and catalase (EC 1.11.1.6) revealed higher levels (29.8, 30.6, 71.4, and 35.3%, respectively) in transgenic seeds than in nontransgenic seeds. Separation of soybean seed proteins was done by two-dimensional polyacrylamide gel electrophoresis, and 192 proteins were identified by matrix-assisted laser desorption/ionization (MALDI) quadrupole time-of-flight (QTOF) mass spectrometry (MS) and electrospray ionization (ESI) QTOF MS. Additionally, the enzyme CP4 EPSPS, involved in the genetic modification, was identified by enzymatic digestions using either trypsin or chymotrypsin and ESI-QTOF MS/MS for identification. From the proteins identified, actin fragment, cytosolic glutamine synthetase, glycinin subunit G1, and glycine-rich RNA-binding protein were shown to be differentially expressed after analysis using the two-dimensional difference gel electrophoresis technique, and applying a regulator factor of 1.5 or greater.

http://www.ncbi.nlm.nih.gov/pubmed/21947011

Agapito-Tenfen, S.; Guerra, M.; Wikmark , O-G; Nodari, R. 2013. Comparative proteomic analysis of genetically modified maize grown under different agroecosystems conditions in Brazil. *Proteome Science*, 11:46.

Bakground: Profiling technologies allow the simultaneous measurement and comparison of thousands of cell components without prior knowledge of their identity. In the present study, we used two-dimensional gel electrophoresis combined with mass spectrometry to evaluate protein expression of Brazilian genetically modified maize hybrid grown under different agroecosystems conditions. To this effect, leaf samples were subjected to comparative analysis using the near-isogenic non-GM hybrid as the comparator.

Results: In the first stage of the analysis, the main sources of variation in the dataset were identified by using Principal Components Analysis which correlated most of the variation to the different agroecosystems conditions. Comparative analysis within each field revealed a total of thirty two differentially expressed proteins between GM and non-GM samples that were identified and their molecular functions were mainly assigned to carbohydrate and energy metabolism, genetic information processing and stress response.

Conclusions: To the best of our knowledge this study represents the first evidence of protein identities with differentially expressed isoforms in Brazilian MON810 genetic background hybrid grown under field conditions. As global databases on outputs from "omics" analysis become available, these could provide a highly desirable benchmark for safety assessments.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4176129/

Parte significativa dos efeitos não desejados observados após transgenia ocorre por pleiotropia[25] (situação em que determinado gene condiciona ou influencia a expressão de mais de uma característica) ou por epistasia/interação gênica (situação contrária à precedente, em que vários genes interagem entre si para influenciar uma única característica). Alguns desses efeitos pleiotrópicos e/ou epistáticos, que tendem a ser ocultados em condições otimizadas de pesquisa, são percebidos apenas quando a planta é submetida a condições ambientais específicas, determinadas a campo por ampla variedade e combinação de fatores de estresses bióticos e abióticos.

Nesse contexto, vários estudos apontam impactos colaterais da transgenia, manifestados pela alteração de diversas vias metabólicas e bioquímicas, em plantas transformadas. Como exemplo, destacamse relações envolvidas no metabolismo da lignina e do nitrogênio.

Escher, N.; Käch, B.; Nentwig, W. 2000. Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber (Crustacea: Isopoda)*. *Basic Appl. Ecol.* 1, 161–169.

Foliage of transgenic maize *Zea mays* L., expressing a Cry1Ab protein derived from *Bacillus thuringiensis* (Berliner) subsp. *kurstaki*, was compared with foliage of the corresponding non-transgenic maize variety in laboratory feeding and decomposition experiments to study the effects of the *B. thuringiensis* protein on the chemical composition of the maize leaves, on the decomposer *Porcellio scaber* (Crustacea: Isopoda), and on leaf-litter-colonising microorganisms. Initial contents of fructose and soluble carbohydrates were significantly higher in non-transgenic maize. Lignin was decomposed more quickly in transgenic maize. Starch, cellulose, hemicellulose and ash content did not differ. Bacterial growth on faeces of *P. scaber* fed on non-transgenic maize was up to 60% higher than on faeces of the transgenic-fed woodlice, but bacterial growth on leaves and fungal growth on faeces were equal on both maize varieties. *P. scaber* showed no significant difference in its consumption rate of transgenic and non-transgenic maize. The number of offspring did not differ between the two treatment groups, but the mortality of juveniles reared on non-transgenic maize leaves was significantly higher. During the first 131 days weight increase of the offspring was significantly higher in the non-transgenic group, but weight increase of adult *P. scaber* was higher in the transgenic group. Due to a slightly lower C:N ratio, a lower lignin

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[25] Quanto à importância biológica da pleiotropia, recomendamos trabalho de Wanga et al., 2010 (*Genomic patterns of pleiotropy and the evolution of complexi*ty. www.pnas.org/cgi/doi/10.1073/pnas.1004666107), disponível em http://www.pnas.org/content/107/42/18034.full

content, and a higher content of soluble carbohydrates, the nutritional quality of transgenic maize leaves was better than that of the non-transgenic variety. This explains the lower mortality of *P. scaber* offspring and the faster weight gain of adult *P. scaber*on the transgenic diet.

http://www.sciencedirect.com/science/article/pii/S1439179104700213

Saxena, D.; Stotzky, G. 2001. Bt corn has a higher lignin content than non-Bt corn. *American Journal of Botany,* 88: 1704-1706.

Bt corn has been genetically modified to express the Cry1Ab protein of Bacillus thuringiensis to kill lepidopteran pests. Fluorescence microscopy and staining with toluidine blue indicated a higher content of lignin in the vascular bundle sheaths and in the sclerenchyma cells surrounding the vascular bundle in all ten Bt corn hybrids, representing three different transformation events, studied than of their respective non-Bt isolines. Chemical analysis confirmed that the lignin content of all hybrids of Bt corn, whether grown in a plant growth room or in the field, was significantly higher (33-97% higher) than that of their respective non-Bt isolines. As lignin is a major structural component of plant cells, modifications in lignin content may have ecological implications.

Artigo completo disponível em http://www.amjbot.org/content/88/9/1704.long

Stotzky, G. 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil,* 266: 77–89.

Insecticidal proteins produced by various subspecies (*kurstaki, tenebrionis*, and *israelensis*) of*Bacillus thuringiensis* (*Bt*) bound rapidly and tightly on clays, both pure mined clay minerals and soil clays, on humic acids extracted from soil, and on complexes of clay and humic acids. Binding reduced susceptibility of the proteins to microbial degradation. However, bound proteins retained biological activity. Purified Cry1Ab protein and protein released from biomass of transgenic *Bt* corn and in root exudates of growing *Bt* corn (13 hybrids representing three transformation events) exhibited binding and persistence in soil. Insecticidal protein was also released in root exudates of*Bt* potato (Cry3A protein) and rice (Cry1Ab protein) but not in root exudates of *Bt* canola, cotton, and tobacco (Cry1Ac protein). Vertical movement of Cry1Ab protein, either purified or in root exudates or biomass of *Bt* corn, decreased as the concentration of the clay minerals, kaolinite or montmorillonite, in soil increased. Biomass of transgenic *Bt* corn decomposed less in soil than biomass of near-isogenic non-*Bt* corn, possibly because biomass of *Bt* corn had a significantly higher content of lignin than biomass of non-*Bt* corn. Biomass of *Bt* canola, cotton, potato, rice, and tobacco also decomposed less than biomass of the respective near-isogenic non-*Bt* plants. However, the lignin content of these *Bt* plants, which was significantly less than that of *Bt* corn, was not significantly different from that of their near-isogenic non-*Bt* counterparts, although it was consistently higher. The Cry1Ab protein had no consistent effects on organisms (earthworms, nematodes, protozoa, bacteria, fungi) in soil or *in vitro*. The Cry1Ab protein was not taken up from soil by non-*Bt* corn, carrot, radish, or turnip grown in soil in which *Bt* corn had been grown or into which biomass of *Bt* corn had been incorporated.

http://link.springer.com/article/10.1007%2Fs11104-005-5945-6

Flores, S.; Saxena, D.; Stotzky, G. 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology & Biochemistry*, 37: 1073-1082.

Bt plants are plants that have been genetically modified to express the insecticidal proteins (e.g. Cry1Ab, Cry1Ac, Cry3A) from subspecies of the bacterium, *Bacillus thuringiensis* (*Bt*), to kill lepidopteran pests that feed on corn, rice, tobacco, canola, and cotton and coleopteran pests that feed on potato. The biomass of these transgenic *Bt* plants (*Bt*+) was decomposed less in soil than the biomass of their near-isogenic non-*Bt*plant counterparts (*Bt*–). Soil was amended with 0.5, 1, or 2% (wt wt^{-1}) ground, dried (50 °C) leaves or stems of *Bt* corn plants; with 0.5% (wt wt^{-1}) ground, dried biomass of *Bt* rice, tobacco, canola, cotton, and potato plants; with biomass of the near-isogenic plants without the respective *cry* genes; or not amended. The gross metabolic activity of the soil was determined by CO_2 evolution. The amounts of C evolved as CO_2 were significantly lower from soil microcosms amended with biomass of *Bt* plants than of non-*Bt* plants. This difference occurred with stems and leaves from two hybrids of *Bt* corn, one of which had a higher C:N ratio than its near-isogenic non-*Bt* counterpart and the other which had essentially the same C:N ratio, even when glucose, nitrogen (NH_4NO_3), or glucose plus nitrogen were added with the biomass. The C:N ratios of the other *Bt* plants (including two other hybrids of *Bt* corn) and their near-isogenic non-*Bt* counterparts were also not related to their relative biodegradation. *Bt* corn had a significantly higher lignin content than near-isogenic non-*Bt* corn. However, the lignin content of the other *Bt* plants, which was significantly lower than that of both*Bt* and non-*Bt* corn, was generally not statistically significantly different, although 10–66% higher, from that of their respective non-*Bt* near-isolines. The numbers of culturable bacteria and fungi and the activity of representative enzymes involved in the degradation of plant biomass were not significantly different between soil amended with biomass of *Bt* or non-*Bt* corn. The degradation of the biomass of all *Bt* plants in the absence of soil but inoculated with a microbial suspension from the same soil was also significantly less than that of their respective inoculated non-*Bt* plants. The addition of streptomycin, cycloheximide, or both to the soil suspension did not alter the relative degradation of *Bt*+ and *Bt*– biomass, suggesting that differences in the soil microbiota were not responsible for the differential decomposition of *Bt*+ and *Bt*– biomass. All samples of soil amended with biomass of *Bt* plants were immunologically positive for the respective Cry proteins and toxic to the larvae of the tobacco hornworm (*Manduca sexta*), which was used as a representative lepidopteran in insect bioassays (no insecticidal assay was done for the Cry3A protein from potato). The ecological and environmental relevance of these findings is not clear.

http://www.sciencedirect.com/science/article/pii/S0038071704004201

Poerschmann, J.; Gathmann, A.; Augustin, J.; Langer, U.; Górecki, T. 2005. Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize - Characterization of lignin patterns. *Journal of Environmental Quality,* 34: 1508-1518.

Transformation of crops, including maize (Zea mays L.), with the cry1Ab gene from Bacillus thuringiensis to combat lepidopteran pests results in pleiotropic effects regarding lignin biosynthesis. Lignin patterns in stems and leaves of two genetically modified Bt-maize varieties (Novelis T and Valmont T) were studied along with their non-Bt near-isolines (Nobilis and Prelude, respectively). Molecular-level based thermochemolysis using tetramethylammonium hydroxide (TMAH) in combination with gas chromatography-mass spectrometry (GC-MS) was used to quantitate the total lignin contents and to identify monomeric lignin subunits including p-hydroxyphenyl (P), guaiacyl (G), and syringyl (S) moieties. The results were supplemented and confirmed by cupric oxide oxidation. The stems of the transgenic lines had higher concentrations of total lignin than the respective isogenic lines: Valmont T/Prelude by 18% and Novelis T/Nobilis by 28%. In contrast, differences in the total lignin concentration of leaves between the transgenic and the respective near-isogenic lines were marginal. There were significant modifications in the ratio of p-hydroxyphenyl/guaiacyl/syringyl molecular marker units of stem lignin between transgenic and isogenic lines. The guaiacyl units (in particular the G18 marker) accounted chiefly for the higher total lignin contents in the transgenic lines. The leaf lignin patterns did not show significant differences in molecular markers between isogenic and transgenic lines. TMAH-induced thermochemolysis--conducted in both the on-line and off-line modes--provided detailed information on the molecular composition of lignin, thus proving superior to the established “wet chemistry” methods of lignin determination.

http://www.ncbi.nlm.nih.gov/pubmed/16091603

Manetti, C.; Bianchetti, C.; Casciani, L.; Castro, C.; Di Cocco, M.; Miccheli, A.; Motto, M.; Conti, F. 2006. A metabonomic study of transgenic maize (*Zea mays*) seeds revealed variations in osmolytes and branched amino acids. *Journal of Experimental Botany*, 57 (11):2613-2625.

The aim of the research was to investigate metabolic variations associated with genetic modifications in the grains of Zea mays using metabonomic techniques. With this in mind, the non-targeted characteristic of the technique is useful to identify metabolites peculiar to the genetic modification and initially undefined. The results obtained showed that the genetic modification, introducing Cry1Ab gene expression, induces metabolic variations involving the primary nitrogen pathway. Concerning the methodological aspects, the experimental protocol used has been applied in this field for the first time. It consists of a combination of partial least square-discriminant analysis and principal component analysis. The most important metabolites for discrimination were selected and the metabolic correlations linking them are identified. Principal component analysis on selected signals confirms metabolic variations, highlighting important details about the changes induced on the metabolic network by the presence of a Bt transgene in the maize genome.

Artigo completo disponível em http://jxb.oxfordjournals.org/content/57/11/2613.long

Têm sido registradas – em plantas geneticamente modificadas e, comparativamente, em seus isogênicos – alterações composicionais e nutricionais relevantes (nos seus teores ou suas biodisponibilidades), que podem ser interpretadas como consequências de perturbações em vias metabólicas e bioquímicas, decorrentes das inserções.

Masoero, F.; Moschini, M.; Rossi, F.; Prandini, A.; Pietri, A. 1999. Nutritive value, mycotoxin contamination and in vitro rumen fermentation of normal and genetically modified corn (cry1A(b)) grown in northern Italy. *Journal Maydica,* Volume: 44 Issue: 3 Pages: 205-209.

An assessment was made on the effect of inserting the cry1A(b) gene of *Bacillus thuringiensis* (Bt) into the genome of two maize hybrids (the newly-developed hybrid from Cargill Semences identified as CR and the traditional hybrid B73 × Mo17) on the analytical composition, the *in vitro* rumen degradability and the mycotoxin contamination of the plant. Transgenicity changed the plant chemical composition as a function of the recipient genotype: starch was increased in the CR-Bt^+ plant (73.3 vs. 70.4%, $P<0.10$), whereas higher lignin content (7.3 vs. 6.3%, $P<0.05$) and lower protein (7.1 vs. 7.7%, $P<0.10$) and soluble nitrogen (26.9 vs. 34.8%, $P<0.10$) contents were observed in the B73 × Mo17-Bt^+ plants in comparison with CR-Bt^+ plants. When not considering the hybrid pedigree, there was a tendency ($P<0.1$) towards a lower protein content in the Bt^+ maize seeds (8.2 vs. 9.2%) and a higher sugar content in stalk and leaves (5.7 vs. 2.9%). The stover degradation increased in the CR-Bt^+variety, probably as the consequence of the higher content of lower structured carbohydrates. Transgenic plants had less ergosterol and fumonisin content than standard maize, suggesting a reduced susceptibility to mould attack.

http://www.cabdirect.org/abstracts/20001605841.html;jsessionid=4F3B4B50CE7AA7EC6E6E-391AE1C34917

Lappe, M.; Bailey, E.; Childress, C.; Setchell, K. 1999. Alterations in clinically important phytoestrogens in genetically modified, herbicide-tolerant soybeans. *Journal of Medicinal Food,* 1, 241-245.

The growing clinical interest and use of soybean-based food products or extracts to increase dietary phytoestrogen intake makes the precise composition of the key biologically active ingredients of soybeans, notably genistin and daidzin of substantial medical interest. Conventional soybeans are increasingly being replaced by genetically modified varieties. We analyzed the phytoestrogen concentrations in two varieties of genetically modified herbicide tolerant soybeans and their isogenic conventional counterparts grown under similar conditions. An overall reduction in phytoestrogen levels of 12-14 percent was observed in the genetically altered soybean strains. Most of this reduction was attributable to reductions in genistin and to a lesser extent daidzin levels, which were significantly lower in modified compared to conventional soybeans in both strains. Significant sample to sample variability in these two phytoestrogens, but not glycitin, was evident in different batches of genetically altered soybeans. Given the high biological potency of isoflavones and their metabolic conversion products, these data suggest genetically modified soybeans may be less potent sources of clinically relevant phytoestrogens than their conventional precursors. These observations, if confirmed in other soybean varieties, heighten the importance of establishing baselines of expected isoflavone levels in transgenic and conventional soy products to ensure uniformity of clinical results. Disclosure of the origins and isoflavone composition of soy food products would be a valuable adjunct to clinical decision-making.

Artigo completo disponível em http://www.mindfully.org/GE/Phytoestrogen-Alteration-GM-Soybeans1jul99.htm

Shewmaker, C.; Sheehy, J.; Daley, M.; Colburn, S.; Ke, D. 1999. Seed-specific overexpression of phytoene synthase: Increase in carotenoids and other metabolic effects. *The Plant Journal*, 20(4): 401–412X.

A bacterial phytoene synthase (crtB) gene was overexpressed in a seed-specific manner and the protein product targeted to the plastid in Brassica napus (canola). The resultant embryos from these transgenic plants were visibly orange and the mature seed contained up to a 50-fold increase in carotenoids. The predominant carotenoids accumulating in the seeds of the transgenic plants were alpha and beta-carotene. Other precursors such as phytoene were also detected. Lutein, the predominant carotenoid in control seeds, was not substantially increased in the transgenics. The total amount of carotenoids in these seeds is now equivalent to or greater than those seen in the mesocarp of oil palm. Other metabolites in the isoprenoid pathway were examined in these seeds. Sterol levels remained essentially the same, while tocopherol levels decreased significantly as compared to non-transgenic controls. Chlorophyll levels were also reduced in developing transgenic seed. Additionally, the fatty acyl composition was altered with the transgenic seeds having a relatively higher percentage of the 18 : 1 (oleic acid) component and a decreased percentage of the 18 : 2 (linoleic acid) and 18 : 3 (linolenic acid) components. This dramatic increase in flux through the carotenoid pathway and the other metabolic effects are discussed.

http://www.ncbi.nlm.nih.gov/pubmed/10607293

Zhou, J. ; Ma, C. ; Xu, H. ; *et al*. 2009. Metabolic profiling of transgenic rice with cryIAc and sck genes: an evaluation of unintended effects at metabolic level by using GC-FID and GC-MS. *J Chromatogr B Analyt Technol Biomed Life Sci*, 877(8-9): 725-732.

The cryIAc and sck genes were introduced to the rice for the purpose of improving the insect resistance. Metabolic profiles of wild and transgenic rice were compared to assess the unintended effects related to gene modification. Wild samples with different sowing dates or sites were also examined to determine the environmental effects on metabolites. The polar compounds of grains were extracted, trimethylsilylated and analyzed by gas chromatography-flame ionization detection (GC-FID). Partial least squares-discriminant analysis (PLS-DA) and principal component analysis (PCA) were applied to differentiate transgenic and wild rice grains. The significantly distinguishable metabolites were picked out, and then identified by gas chromatography-mass spectrometry (GC-MS). It was found that both the environment and gene manipulation had remarkable impacts on the contents of glycerol-3-phosphate, citric acid, linoleic acid, oleic acid, hexadecanoic acid, 2,3-dihydroxypropyl ester, sucrose, 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester and so on. Sucrose, mannitol and glutamic acid had a significant increase in transgenic grains in contrast to those in non-genetically modified (GM) rice.

http://www.ncbi.nlm.nih.gov/pubmed/19233746

Jiao, Z.; Si, X.; Li, G.; Zhang, Z.; Xu, X. 2010. Unintended compositional changes in transgenic rice seeds (*Oryza sativa L.*) studied by spectral and chromatographic analysis coupled with chemometrics methods. *J. Agric. Food Chem.*, 58, 1746-1754.

Unintended compositional changes in transgenic rice seeds were studied by near-infrared reflectance, GC-MS, HPLC, and ICP-AES coupled with chemometrics strategies. Three kinds of transgenic rice with resistance to fungal diseases or insect pests were comparatively studied with the nontransgenic counterparts in terms of key nutrients such as protein, amino acids, fatty acids, vitamins, elements, and antinutrient phytic acid recommended by the Organization for Economic Co-operation and Development (OECD). The compositional profiles were discriminated by chemometrics methods, and the discriminatory compounds were protein, three amino acids, two fatty acids, two vitamins, and several elements. Significance of differences for these compounds was proved by analysis of variance, and the variation extent ranged from 20 to 74% for amino acids, from 19 to 38% for fatty acids, from 25 to 57% for vitamins, from 20 to 50% for elements, and 25% for protein, whereas phytic acid content did not change significantly. The unintended compositional alterations as well as unintended change of physical characteristic in transgenic rice compared with nontransgenic rice might be related to the genetic transformation, the effect of which needs to be elucidated by additional studies.

http://www.ncbi.nlm.nih.gov/pubmed/20050687

Abdo, E. ; Barbary O.; Shaltout O. 2013. Chemical Analysis of Bt corn "Monsanto810: Ajeeb-YG" and its counterpart non-Bt corn "Ajeeb". *IOSR Journal of Applied Chemistry*, Vol 4 Issue 1, pp55-60.

Commercialization of biotech crops has started since 1996, where the cultivated area of these crops was increased from 1.7 million hectares in 1996 to 170.3 million hectares in 2012 according to the latest statistics in 2012. Bt corn "MON810: Ajeeb YG®" is one of these crops that express endotoxin from Bacillus thuringiensis (Bt) throughout the whole plant. This study was designed to assess the safety of Bt corn by comparing its compositional chemical analysis with its conventional counterpart "Ajeeb". Moisture content, crude fat, total saccharides, starch, & crude fiber were determined; sodium, potassium, magnesium, calcium and phosphorous content were measured, tannins & phytic acid were determined as anti-nutrients. Amino acids and fatty acids profiles were also evaluated. Results indicated the presence of significant differences between both of Bt corn and its counterpart.

Artigo completo disponível em http://www.academia.edu/3341205/Chemical_Analysis_of_Bt_corn_Mon-810_Ajeeb-YG_and_its_counterpart_non-Bt_corn_Ajeeb_

Li, S.; Chen, L.; Zheng, F.; Li, Y. 2013. Effect of the cp4-epsps gene on metal bioavailability in maize and soybean using bionic gastrointestinal tracts and ICP-MS determination. *J. Agric. Food Chem.,* 20;61(7):1579-84. doi: 10.1021/jf303962a.

The transformation and metabolism of dietary compounds are affected significantly by gut microbiota. Hence, gut microbiota are used to improve bionic gastrointestinal tracts. The effect of the cp4-epsps gene on metal bioavailability was proved by the comparison of the affinity-liposome metal content ratio (AMCR) in transgenic and conventional crops. The bioavailability of V, Mn, Co, Ga, Ag, Ba, and Pb in roundup ready soybean decreased significantly because the ratio of AMCR (R(AMCR)) in the transgenic crop and its corresponding conventional type ranged from 0.36 to 0.69. In roundup ready maize, metal bioavailability decreased for Li and Cr (i.e., R(AMCR) was 0.26 and 0.39, respectively) but increased for V, Co, and Pb (i.e., R(AMCR) was 1.48, 2.07, and 2.12, respectively). Compared with conventional crops, safe dosage and maximum consumption of roundup ready crops were 1.59 times for soybean and 0.78 times for maize.

http://pubs.acs.org/doi/abs/10.1021/jf303962a

As consequências das perturbações metabólicas e bioquímicas observadas em plantas transgênicas nem sempre podem ser completamente caracterizadas. Alterações em vias metabólicas são de difícil previsão e podem afetar características agronômicas importantes, associadas à produtividade.

Preeti, R.; Singh, A.; Ray, K.; Chaudhary, B.; et al. 2011. Detrimental effect of expression of Bt endotoxin Cry1Ac on in vitro regeneration, in vivo growth and development of tobacco and cotton transgenics. *Journal of Biosciences*, 36(2), 363-376.

High levels of expression of the cry1Ac gene from Bacillus thuringiensis cannot be routinely achieved in transgenic plants despite modifications made in the gene to improve its expression. This has been attributed to the instability of the transcript in a few reports. In the present study, based on the genetic transformation of cotton and tobacco, we show that the expression of the Cry1Ac endotoxin has detrimental effects on both the in vitro and in vivo growth and development of transgenic plants. A number of experiments on developing transgenics in cotton with different versions of cry1Ac gene showed that the majority of the plants did not express any Cry1Ac protein. Based on Southern blot analysis, it was also observed that a substantial number of lines did not contain the cry1Ac gene cassette although they contained the marker gene nptII. More significantly, all the lines that showed appreciable levels of expression were found to be phenotypically abnormal. Experiments on transformation of tobacco with different constructs expressing the cry1Ac gene showed that in vitro regeneration was inhibited by the encoded protein. Further, out of a total of 145 independent events generated with the different cry1Ac gene constructs in tobacco, only 21 showed expression of the Cry1Ac protein, confirming observations made in cotton that regenerants that express high levels of the Cry1Ac protein are selected against during regeneration of transformed events. This problem was circumvented by targeting the Cry1Ac protein to the chloroplast, which also significantly improved the expression of the protein.

Artigo completo disponível em http://www.ias.ac.in/jbiosci/jun2011/363.pdf

Powell, A.; Nguyen, C.; Hill, T.; Cheng, K.; et al. 2012. Uniform ripening encodes a Golden 2-like transcription factor regulating tomato fruit chloroplast development. *Science*, vol 336 29.

Modern tomato (Solanum lycopersicum) varieties are bred for uniform ripening (u) light green fruit phenotypes to facilitate harvests of evenly ripened fruit. U encodes a Golden 2-like (GLK) transcription factor, SlGLK2, which determines chlorophyll accumulation and distribution in developing fruit. In tomato, two GLKs--SlGLK1 and SlGLK2--are expressed in leaves, but only SlGLK2 is expressed in fruit. Expressing GLKs increased the chlorophyll content of fruit, whereas SlGLK2 suppression recapitulated the u mutant phenotype. GLK overexpression enhanced fruit photosynthesis gene expression and chloroplast development, leading to elevated carbohydrates and carotenoids in ripe fruit. SlGLK2 influences photosynthesis in developing fruit, contributing to mature fruit characteristics and suggesting that selection of u inadvertently compromised ripe fruit quality in exchange for desirable production traits.

http://www.ncbi.nlm.nih.gov/pubmed/22745430

Também, a ativação de novas vias metabólicas ou a síntese de novos subprodutos mediante organismos transgênicos podem representar novos espectros de riscos biológicos potenciais, tanto para a saúde humana e animal como para organismos não alvo.

Donegan, K.K., Palm, C.J., Fieland, V.J.,et al. Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the Bacillus thuringiensis var. kurstaki endotoxin. *Applied soil ecology*., 1995;2(2):111-124.

An important aspect of the risk assessment of pesticidal transgenic plants is the potential for detrimental effects on the soil ecosystem from residual plant material following harvesting and tillage. We evaluated this concern by placing leaves of three different lines of cotton genetically engineered to produce the *Bacillus thuringiensis* var. *kurstaki* (B.t.k.) endotoxin in soil and monitoring numbers and species of indigenous soil bacteria and fungi. Four experiments, lasting 28 or 56 days, were performed using combinations of the following treatments: (1) soil only; (2) soil +purified B.t.k. toxin; (3) soil +parental cotton; (4) soil +purified B.t.k. toxin +parental cotton; (5) soil + B.t.k. toxin-producing cotton.

Two of the three transgenic cotton lines caused a transient increase in total bacterial and fungal population levels that was significantly higher on several sample days in the experiments than the levels in the other treatments. In contrast, neither the third transgenic cotton line nor the purified B.t.k. toxins had any significant effects on the total numbers of bacteria and fungi.

Transient changes in bacterial species composition, measured by biochemical tests of individual cultures, community substrate utilization and DNA fingerprinting, were also observed in treatments with the two transgenic plant lines.

The plant line specificity of the response, and the lack of effects from the purified B.t.k. toxins, suggest that the observed effects of the two transgenic plant lines on soil microorganisms may not have resulted from the plants' production of B.t.k. toxin. We suggest that genetic manipulation or tissue culturing of the plants may have produced a change in plant characteristics, aside from B.t.k. toxin production, that can influence growth and species composition of soil microorganisms.

http://www.sciencedirect.com/science/article/pii/0929139394000437

Schrøder, M.; Poulsen, M.; Wilcks, A.; et al. 2007. A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. *Food Chem Toxicol.,* 45(3): 339-349.

An animal model for safety assessment of genetically modified foods was tested as part of the SAFOTEST project. In a 90-day feeding study on Wistar rats, the transgenic KMD1 rice expressing Cry1Ab protein was compared to its non-transgenic parental wild type, Xiushui 11. The KMD1 rice contained 15mg Bt toxin/kg and based on the average feed consumption the daily intake was 0.54mg Bt toxin/kg body weight. No adverse effects on animal behaviour or weight gain were observed during the study. Blood samples collected one week prior to sacrifice were analyzed and compared for standard haematological and biochemical parameters. A few parameters were significantly different, but all within the normal reference intervals for rats of this breed and age and not in relation to any other findings, thus not considered treatment related. Upon sacrifice a large number of organs were weighed, macroscopic and histopathological examinations were performed with only minor changes to report. The aim of the study was to use a known animal model in performance of safety assessment of a GM crop, in this case KMD1 rice. The results show no adverse or toxic effects of KMD1 rice when tested in the design used in this 90-day study. Nevertheless the experiences from this study lead to the overall conclusion that safety assessment for unintended effects of a GM crop cannot be done without additional test group(s).

http://www.ncbi.nlm.nih.gov/pubmed/17050059

Zolla, L.; Rinalducci, S.; Antonioli, P.; Righetti, P. 2008. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. *Journal of Proteome Research,* 7, 1850-1861.

To improve the probability of detecting unintended side effects during maize gene manipulations by bombardment, proteomics was used as an analytical tool complementary to the existing safety assessment techniques. Since seed proteome is highly dynamic, depending on the species variability and environmental influence, we analyzed the proteomic profiles of one transgenic maize variety (event MON 810) in two subsequent generations (T05 and T06) with their respective isogenic controls (WT05 and WT06). Thus, by comparing the proteomic profiles of WT05 with WT06 we could determine the environmental effects, while the comparison between WT06 and T06 seeds from plants grown under controlled conditions enabled us to investigate the effects of DNA manipulation. Finally, by comparison of T05 with T06 seed proteomes, it was possible to get some indications about similarities and differences between the adaptations of transgenic and isogenic plants to the same strictly controlled growth environment. Approximately 100 total proteins resulted differentially modulated in the expression level as a consequence of the environmental influence (WT06 vs WT05), whereas 43 proteins resulted up- or down-regulated in transgenic seeds with respect to their controls (T06 vs WT06), which could be specifically related to the insertion of a single gene into a maize genome by particle bombardment. Transgenic seeds responded differentially to the same environment as compared to their respective isogenic controls, as a result of the genome rearrangement derived from gene insertion. To conclude, an exhaustive differential proteomic analysis allows to determine similarities and differences between traditional food and new products (substantial equivalence), and a case-by-case assessment of the new food should be carried out in order to have a wide knowledge of its features.

Artigo completo disponível em http://www.ufpe.br/biolmol/Tec-mol-biol/papers-TMB-2010/proteomica-Zolla_et_al-2008.pdf

Bortolotto, O.; Silva G.; Bueno, A.; Pomari, A.; Martinelli, S.; Head, G.; Carvalho, R.; Barbosa, G. 2014. Development and reproduction of *Spodoptera eridania* (*Lepidoptera: Noctuidae*) and its egg parasitoid *Telenomus remus* (*Hymenoptera: Platygastridae*) on the genetically modified soybean (Bt) MON 87701 × MON 89788. *Bulletin of Entomological Research,* vol 104, Issue 06, pp 724-730.

Genetically modified crops with insect resistance genes from Bacillus thuringiensis Berliner (Bt-plants) are increasingly being cultivated worldwide. Therefore, it is critical to improve our knowledge of their direct or indirect impact not only on target pests but also on non-target arthropods. Hence, this study evaluates comparative leaf consumption and performance of Spodoptera eridania (Cramer), a species that is tolerant of the Cry1Ac protein, fed with Bt soybean, MON 87701×MON 89788 or its non-Bt isoline. We also assessed the comparative performance of the egg parasitoid Telenomus remus Nixon on eggs of S. eridania produced from individuals that fed on these two soybean isolines as larvae. Results showed that Bt soybean reduced by 2 days larval development and increased by 3 days adult male longevity. Therefore, we conclude that the effect of Bt soybean MON 87701×MON 89788 on S. eridania development and reproduction is small, and favorable to pest development. These differences are less likely to directly result from the toxin presence but indirectly from unintended changes in plant characteristics caused by the insertion of the transgene. Our results should be viewed as an alert that S. eridania populations may increase in Bt soybeans, but on the other hand, no adverse effects of this technology were observed for the egg parasitoid T. remus which can help to prevent S. eridania outbreaks on these crops.

http://www.ncbi.nlm.nih.gov/pubmed/25248849

2.4 Eventos piramidados: rumo ao reducionismo científico

A designação “evento piramidado” (também chamado de “evento estaqueado” ou “empilhado”) passou a ser usada para caracterizar plantas transgênicas obtidas por cruzamento convencional envolvendo dois ou mais eventos transgênicos do tipo simples (com um transgene). Duplos, triplos ou n-piramidados (com n transgênicos) são chamados da mesma forma. Já desde a última meia década, a quase totalidade das “novas” plantas transgênicas liberadas comercialmente correspondem a eventos piramidados, que, em alguns casos, comportam mais de cinco transgenes.

Evidentemente a complexidade das interações é crescente, com a multiplicação de fatores de risco. Por esse motivo, para evitar processos de avaliação difíceis e onerosos – e consequentemente facilitar a liberação comercial de plantas transgênicas – os órgãos reguladores, apoiados pela indústria, passaram a adotar alternativas de avaliação que merecem discussão detalhada. Privilegiando aspectos de praticidade e à revelia de ajustamento de conduta a preceitos de base científica, passaram a utilizar abordagem assentada no pressuposto de que fenômenos percebidos de forma isolada não se alterariam quando obrigados a manifestação conjunta. Em outras palavras, os efeitos observados em cada evento individual apresentariam manifestação meramente aditiva nos piramidados, inexistindo a possibilidade de interações que pudessem levar a manifestações inesperadas, inexistentes nos eventos simples. Ou seja: se os eventos transgênicos A e B isoladamente não apresentam riscos, então o novo evento AxB, a priori, não apresentaria riscos.

Desse modo, e no interesse das empresas, estudos aplicados aos casos isolados, que negam possibilidade de danos, passaram a servir de base para atestar a segurança dos eventos múltiplos. Assim, em que pesem os riscos para a sociedade, eventos mais complexos, em vez de avaliações completas, passaram a ser julgados com base em análises expeditas.

Ora, isso reduziu as possibilidades de compreensão dos eventos piramidados, ampliando faixas de risco associadas à utilização da tecnologia. Ainda assim, em que pese o número extremamente reduzido de pesquisas aplicadas aos piramidados, a literatura científica registra estudos que apontam para efeitos inesperados, resultantes do estaqueamento de transgenes.

Mais grave do que isso é o fato de que, ao mesmo tempo em que as interações gênicas – entre transgenes – são pouco conhecidas, é praticamente nulo o conhecimento sobre suas consequências potenciais no epigenoma dos novos organismos.

Londo, J.; Bollman, M.; Sagers, C.; Lee, E.; Watrud, L. 2011. Changes in fitness-associated traits due to the stacking of transgenic glyphosate resistance and insect resistance in *Brassica napus* L. *Heredity*, 107, 328–337; doi:10.1038/hdy.2011.19.

Increasingly, genetically modified crops are being developed to express multiple ‘stacked’ traits for different types of transgenes, for example, herbicide resistance, insect resistance, crop quality and tolerance to environmental stresses. The release of crops that express multiple traits could result in ecological changes in weedy environments if feral crop plants or hybrids formed with compatible weeds results in more competitive plants outside of agriculture. To examine the effects of combining transgenes, we developed a stacked line of canola (Brassica napus L.) from a segregating F(2) population that expresses both transgenic glyphosate resistance (CP4 EPSPS) and lepidopteran insect resistance (Cry1Ac). Fitness-associated traits were evaluated between this stacked genotype and five other Brassica genotypes in constructed mesocosm plant communities exposed to insect herbivores (Plutella xylostella L.) or glyphosate-drift. Vegetative biomass, seed production and relative fecundity were all reduced in stacked trait plants when compared with non-transgenic plants in control treatments, indicating potential costs of expressing multiple transgenes without selection pressure. Although costs of the transgenes were offset by selective treatment, the stacked genotype continued to produce fewer seeds than either single transgenic line. However, the increase in fitness of the stacked genotype under selective pressure contributed to an increased number of seeds within the mesocosm community carrying unselected, hitchhiking transgenes. These results demonstrate that the stacking of these transgenes in canola results in fitness costs and benefits that are dependent on the type and strength of selection pressure, and could also contribute to changes in plant communities through hitchhiking of unselected traits.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3182500/

Shi, G.; Chavas, J-P.; Lauer, J. 2013. Commercialized transgenic traits, maize productivity and yield risk. *Nature Biotechnology,* vol 31 number 2.

Sem resumo.

Artigo completo disponível em http://www.nature.com/nbt/journal/v31/n2/full/nbt.2496.html

Agapito-Tenfen, S.; Vilperte, V.; Benevenuto, R.; Rover, C.**;** Traavik, T.**;** Nodari, R. 2014. Effect of stacking insecticidal cry and herbicide tolerance *epsps t*ransgenes on transgenic maize proteome. *Plant Biology*, 14:346 Open access.

Background: The safe use of stacked transgenic crops in agriculture requires their environmental and health risk assessment, through which unintended adverse effects are examined prior to their release in the environment. Molecular profiling techniques can be considered useful tools to address emerging biosafety gaps. Here we report the first results of a proteomic profiling coupled to transgene transcript expression analysis of a stacked commercial maize hybrid containing insecticidal and herbicide tolerant traits in comparison to the single event hybrids in the same genetic background.
Results: Our results show that stacked genetically modified (GM) genotypes were clustered together and distant from other genotypes analyzed by PCA. Twenty-two proteins were shown to be differentially modulated in stacked and single GM events versus non-GM isogenic maize and a landrace variety with Brazilian genetic background. Enrichment analysis of these proteins provided insight into two major metabolic pathway alterations: energy/carbohydrate and detoxification metabolism. Furthermore, stacked transgene transcript levels had a significant reduction of about 34% when compared to single event hybrid varieties.
Conclusions: Stacking two transgenic inserts into the genome of one GM maize hybrid variety may impact the overall expression of endogenous genes. Observed protein changes differ significantly from those of single event lines and a conventional counterpart. Some of the protein modulation did not fall within the range of the natural variability for the landrace used in this study. Higher expression levels of proteins related to the energy/carbohydrate metabolism suggest that the energetic homeostasis in stacked versus single event hybrid varieties also differ. Upcoming global databases on outputs from ¿omics¿ analyses could provide a highly desirable benchmark for the safety assessment of stacked transgenic crop events. Accordingly, further studies should be conducted in order to address the biological relevance and implications of such changes.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4273480/

Cabe ressaltar que a avaliação de riscos associados a eventos estaqueados deve considerar tanto possibilidades de interações entre os produtos de expressão dos transgenes como entre os agrotóxicos a eles sistematicamente associados (herbicidas, por exemplo). Discussões restritas ao exame de ligações e interações diretas, entre os transgenes propriamente ditos, além de insuficientes, são por demais limitadas em termos de abrangência, para oferecer segurança mínima ao consumo e ao ambiente.

Reconhecidamente, os produtos de expressão da transgenia interagem com fatores ambientais, podendo resultar em efeitos distintos daqueles esperados ou mesmos observados de forma isolada. Além disso, dificuldades de simulação e projeção impedem que – com base em informações coletadas na ausência daquelas combinações – se realizem inferências adequadas sobre possíveis impactos relacionados à escala de adoção e à sinergia de produtos dessas tecnologias.

Accinelli, C.; Screpanti, C; Vicari, A.; Catizone, P. 2004. Influence of insecticidal toxins from *Bacillus thuringiensis* subsp. *kurstaki* on the degradation of glyphosate and glufosinate-ammonium in soil samples. *Agriculture, Ecosystems and Environment,* 103 497–507.

Investigations dealing with the persistence in soil of glyphosate [*N*-(phosphonomethyl) glycine] (GLYP) and glufosinate-ammonium [the ammonium salt of DL-homoalanin-4-yl(methyl)phosphinic acid] (GLUF) herbicides and of insecticidal toxins produced by *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) are largely reported in the literature. However, no information on the influence of these insecticidal toxins on the persistence in soil of herbicides is available. Preliminary results regarding the influence of insecticidal toxins extracted from a commercial formulation of *B. thuringiensis* subsp. *kurstaki* (Berliner) (Btk) on the degradation of the herbicides glyphosate and glufosinate-ammonium in a loam and a sandy loam soil, under laboratory conditions, were obtained. Soil microbial carbon (SMC) and insecticidal activity of incubated soil samples were also estimated. In both soil types, persistence of GLYP was significantly higher with respect to GLUF. Average GLYP and GLUF half-life was 14.4 and 8.0 days, respectively. Addition of Btk toxins lead to a significant increase of GLYP and GLUP persistence in both soil types. More specifically, average GLYP and GLUF half-life in soil samples receiving the Btk treatment was 24.3 and 14.2 days, respectively. In contrast to herbicide persistence in soil, Btk toxins did not influence microbial carbon content of incubated soil samples. The insecticidal activity of Btk toxins in soil rapidly decreased during the 28-day incubation time. Considering that degradation of GLYP and GLUF was mainly a microbial process, the absence of effects of Btk toxins on the soil microbial carbon and the rapid decrease of insecticidal activity of Btk toxins in the soil suggest a possible effect of the Btk toxins on other soil properties and/or mechanisms influencing herbicide degradation. The present preliminary investigation permitted to highlight the possibility of the Btk toxins to enhance the persistence of GLYP and GLUF in soil, under laboratory conditions. However, further studies are necessary to investigate whether or not the effects observed in this study under artificial and controlled conditions can be extrapolated to field conditions.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/IITBT.pdf

Mesnage, R.; Clair, E.; Gress, S.; Then, C.; Székács, A.; Séralini, G-H. 2012. Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. *Journal of Applied Toxicology,* DOI 10.1002/jat.2712

The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified *Bt* insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac *Bt* toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspase 3/7 inductions. Cry1Ab caused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000 ppm is necrotic and apoptotic from 50 ppm, far below agricultural dilutions (50% lethal concentration 57.5 ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified *Bt* toxins are not inert on nontarget human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.

Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2012/11/mesnage2011.pdf

3. Recombinações genéticas interespecíficas e escape de transgenes

A possibilidade/capacidade de o transgene inserido em determinado organismo vir a escapar daquele genoma, deslocando-se para o meio ambiente e afetando outros organismos ali estabelecidos, constitui importante fonte de incertezas e riscos.

Tal escape é possível por meio de Transferência Vertical (TVG) ou Horizontal (THG) de Genes. No primeiro caso, o escape ocorre pelo cruzamento sexual entre organismos geneticamente aparentados (via de regra, de uma mesma espécie). Essa modalidade de transferência será discutida em especial na Parte 2 (item 4), onde tratamos de problemas agronômicos associados à transgenia.

No caso da Transferência Horizontal de Genes (THG), o transgene poderá escapar do organismo para o ambiente por meio de interação direta envolvendo o DNA do organismo transgênico e agentes de transporte genético, como vírus, plasmídeos e transposons.

3.1 Transferência horizontal de genes (THG): revolução do conhecimento científico

A Transferência Horizontal de Genes consiste em modalidade de trocas de DNA entre organismos não aparentados, sem o recurso da fecundação. A THG permite trocar informação genética e atua em escala tão importante e frequente que parece constituir um dos motores da evolução das espécies. A THG ocorre de forma contínua e permanentemente no mundo vivo, não distinguindo entre as espécies biológicas.

REV – Koonin, E. 2003. Horizontal gene transfer: the path to maturity. *Molecular Microbiology* 50 (3): 725-27.

Sem resumo.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2958.2003.03808.x/full

Woloszynska, M.; Bocer, T.; Mackiewicz, P.; Janska, H. 2004. A fragment of chloroplast DNA was transferred horizontally, probably from non-eudicots, to mitrochondrial genome of Phaseolus. *Plant Mol Biol.*, 56 (5): 811-20.

The mitochondrial genomes of some Phaseolus species contain a fragment of chloroplast trnA gene intron, named pvs-trnA for its location within the Phaseolus vulgaris sterility sequence (pvs). The purpose of this study was to determine the type of transfer (intracellular or horizontal) that gave rise to pvs-trnA. Using a PCR approach we could not find the respective portion of the trnA gene as a part of pvs outside the Phaseolus genus. However, a BLAST search revealed longer fragments of trnA present in the mitochondrial genomes of some Citrus species, Helianthus annuus and Zea mays. Basing on the identity or near-identity between these mitochondrial sequences and their chloroplast counterparts we concluded that they had relocated from chloroplasts to mitochondria via recent, independent, intracellular DNA transfers. In contrast, pvs-trnA displayed a relatively higher sequence divergence when compared with its chloroplast counterpart from Phaseolus vulgaris. Alignment of pvs-trnA with corresponding trnA fragments from 35 plant species as well as phylogenetic analysis revealed that pvs-trnA grouped with non-eudicot sequences and was well separated from all Fabales sequences. In conclusion, we propose that pvs-trnA arose via horizontal transfer of a trnA intron fragment from chloroplast of a non-eudicot plant to Phaseolus mitochondria. This is the first example of horizontal transfer of a chloroplast sequence to the mitochondrial genome in higher plants.

http://www.ncbi.nlm.nih.gov/pubmed/15803417

Daubin, V.; Ochman, H. 2004. Quartet mapping and the extent of lateral transfer in bacterial genomes. *Mol Biol Evol,* 21 (1): 86-89.

Several recent analyses have used quartet-based methods to assess the congruence among phylogenies derived for large sets of genes from prokaryotic genomes. The principal conclusion from these studies is that lateral gene transfer (LGT) has blurred prokaryotic phylogenies to such a degree that the darwinian scheme of treelike evolution might be abandoned in favor of a net or web. Here, we focus on one of these methods, quartet mapping, and show that its application can lead to overestimation of the extent of inferred LGT in prokaryotes, particularly when applied to distantly related taxa.

Artigo completo disponível em http://mbe.oxfordjournals.org/content/21/1/86.long

Steale, R.; Hampson, S.; Stover, N.; Kibler, D.; Bode, H.; 2004. Probable horizontal transfer between a protist and a cnidarian. *Current Biology,* 14(8): 298-9.

Sem resumo.

Artigo completo disponível em http://www.cell.com/current-biology/fulltext/S0960-9822(04)00224-6

Davis, C.; Wurdack, K. 2004. Host-to-parasite gene transfer in flowering plants: Phylogenetic evidence from Malpighiales. *Science*, 305: 676-78.

Horizontal gene transfer (HGT) between sexually unrelated species has recently been documented for higher plants, but mechanistic explanations for HGTs have remained speculative. We show that a parasitic relationship may facilitate HGT between flowering plants. The endophytic parasites Rafflesiaceae are placed in the diverse order Malpighiales. Our multigene phylogenetic analyses of Malpighiales show that mitochondrial (matR) and nuclear loci (18S ribosomal DNA and PHYC) place Rafflesiaceae in Malpighiales, perhaps near Ochnaceae/Clusiaceae. Mitochondrial nad1B-C, however, groups them within Vitaceae, near their obligate host Tetrastigma. These discordant phylogenetic hypotheses strongly suggest that part of the mitochondrial genome in Rafflesiaceae was acquired via HGT from their hosts.

http://www.ncbi.nlm.nih.gov/pubmed/15256617

REV – Andersson, J. 2005. Lateral gene transfer in eukaryotes. *Cell Mol Life Sci,* 62: 1182–1197.

Lateral gene transfer -- the transfer of genetic material between species -- has been acknowledged as a major mechanism in prokaryotic genome evolution for some time. Recently accumulating data indicate that the process also occurs in the evolution of eukaryotic genomes. However, there are large rate variations between groups of eukaryotes; animals and fungi seem to be largely unaffected, with a few exceptions, while lateral gene transfer frequently occurs in protists with phagotrophic lifestyles, possibly with rates comparable to prokaryotic organisms. Gene transfers often facilitate the acquisition of functions encoded in prokaryotic genomes by eukaryotic organisms, which may enable them to colonize new environments. Transfers between eukaryotes also occur, mainly into larger phagotrophic eukaryotes that ingest eukaryotic cells, but also between plant lineages. These findings have implications for eukaryotic genomic research in general, and studies of the origin and phylogeny of eukaryotes in particular.

http://www.ncbi.nlm.nih.gov/pubmed/15761667

Babić, A.; Lindner, A.; Vulić, M.; Stewart, E.; Radman, M. 2008. Direct Visualization of Horizontal Gene Transfer. *Science*, vol 319, no. 5869 pp. 1533-1536 DOI:10.1126/science.1153498.

Conjugation allows bacteria to acquire genes for antibiotic resistance, novel virulence attributes, and alternative metabolic pathways. Using a fluorescent protein fusion, SeqA-YFP, we have visualized this process in real time and in single cells of Escherichia coli. We found that the F pilus mediates DNA transfer at considerable cell-to-cell distances. Integration of transferred DNA by recombination occurred in up to 96% of recipients; in the remaining cells, the transferred DNA was fully degraded by the RecBCD helicase/nuclease. The acquired integrated DNA was tracked through successive replication rounds and was found to occasionally split and segregate with different chromosomes, leading to the inheritance of different gene clusters within the cell lineage. The incidence of DNA splitting corresponds to about one crossover per cell generation.

Artigo completo disponível em http://www.sciencemag.org/content/319/5869/1533.long

Dana, C.; Glauber, K.; Chan, T.; Bridge, D.; Steele, R. 2012. Incorporation of a Horizontally Transferred Gene into an Operon during Cnidarian Evolution. *PLOS ONE,* 7(2): e31643.

Genome sequencing has revealed examples of horizontally transferred genes, but we still know little about how such genes are incorporated into their host genomes. We have previously reported the identification of a gene (*flp*) that appears to have entered the *Hydra* genome through horizontal transfer. Here we provide additional evidence in support of our original hypothesis that the transfer was from a unicellular organism, and we show that the transfer occurred in an ancestor of two medusozoan cnidarian species. In addition we show that the gene is part of a bicistronic operon in the *Hydra* genome. These findings identify a new animal phylum in which trans-spliced leader addition has led to the formation of operons, and define the requirements for evolution of an operon in *Hydra*. The identification of operons in *Hydra* also provides a tool that can be exploited in the construction of transgenic *Hydra* strains.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0031643

Domingues, S.; Harms, K.; Fricke, W.; Johnsen, P.; Silva, G.; *et al.* 2012. Natural Transformation Facilitates Transfer of Transposons, Integrons and Gene Cassettes between Bacterial Species. *PLoS Pathog,* 8(8): e1002837. doi:10.1371/journal.ppat.1002837.

We have investigated to what extent natural transformation acting on free DNA substrates can facilitate transfer of mobile elements including transposons, integrons and/or gene cassettes between bacterial species. Naturally transformable cells of Acinetobacter baylyi were exposed to DNA from integron-carrying strains of the genera Acinetobacter, Citrobacter, Enterobacter, Escherichia, Pseudomonas, and Salmonella to determine the nature and frequency of transfer. Exposure to the various DNA sources resulted in acquisition of antibiotic resistance traits as well as entire integrons and transposons, over a 24 h exposure period. DNA incorporation was not solely dependent on integrase functions or the genetic relatedness between species. DNA sequence analyses revealed that several mechanisms facilitated stable integration in the recipient genome depending on the nature of the donor DNA; homologous or heterologous recombination and various types of transposition (Tn21-like and IS26-like). Both donor strains and transformed isolates were extensively characterized by antimicrobial susceptibility testing, integron- and cassette-specific PCRs, DNA sequencing, pulsed field gel electrophoreses (PFGE), Southern blot hybridizations, and by re-transformation assays. Two transformant strains were also genome-sequenced. Our data demonstrate that natural transformation facilitates interspecies transfer of genetic elements, suggesting that the transient presence of DNA in the cytoplasm may be sufficient for genomic integration to occur. Our study provides a plausible explanation for why sequence-conserved transposons, IS elements and integrons can be found disseminated among bacterial species. Moreover, natural transformation of integron harboring populations of competent bacteria revealed that interspecies exchange of gene cassettes can be highly efficient, and independent on genetic relatedness between donor and recipient. In conclusion, natural transformation provides a much broader capacity for horizontal acquisitions of genetic elements and hence, resistance traits from divergent species than previously assumed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3410848/pdf/ppat.1002837.pdf

Esse assunto volta a ser discutido no item 4.2. da Parte 4, com artigos que examinam a possibilidade de transferência horizontal de (trans)genes em células de mamíferos e/ou micro-organismos simbiontes de mamíferos (bactérias do sistema digestivo e/ou da cavidade bucal em especial), e no item 3.3 da Parte 3, onde estão reunidos artigos que tratam da possibilidade de THG entre organismos do meio ambiente em geral e organismos transgênicos.

3.2 Preocupações específicas com o promotor P35S CaMv

Existem aspectos de biossegurança não bem trabalhados nas análises de risco convencionais, relativamente a elementos da construção genética que – além do transgene de interesse – contêm informações necessárias à expressão do transgene nos organismos transformados. Um desses elementos é o promotor originário do vírus do mosaico da couve-flor (CaMV) e está associado a quase todas as construções genéticas inseridas em plantas GM comercializadas. Denominado P35S CaMv, esse elemento carrega a possibilidade de interagir com outros vírus vegetais naturais e gerar, assim, novos vírus. Além disso, pode ativar o CaMV em espécies de plantas normalmente não suscetíveis aquela doença.

Esses riscos, cujas consequências agronômicas devem ser consideradas relevantes, têm sido motivo de alertas já há quase duas décadas por pesquisadores independentes.

Gal, S.; Pisan, B.; Hohn, T.; Grimsley, N; Hohn, B. 1992. Agroinfection of transgenic plants leads to viable cauliflower mosaic virus by intermolecular recombination. *Virology,* 187, 525–533.

Intermolecular reconstitution of a plant virus has been detected in whole plants in a system using a defective cauliflower mosaic virus genome and transgenic host plants containing the missing viral gene. The information for the gene VI protein of the virus was integrated into the chromosome of host Brassica napus plants and leaves of these plants were inoculated with Agrobacterium tumefaciens containing the complementing viral sequences. In several cases, upper leaves contained replicating viral DNA which was able to incite CaMV symptoms on turnip plants. The sequence of the resultant recombinant viral molecules suggested that both DNA and RNA recombination events may have been involved in the production of functional virus, one event being gene targeting of the T-DNA.

http://www.ncbi.nlm.nih.gov/pubmed/1546451

Wintermantel, W.; Schoelz, J. 1996. Isolation of Recombinant Viruses between Cauliflower Mosaic Virus and a Viral Gene in Transgenic Plants under Conditions of Moderate Selection Pressure. *Virology*, 223, 156–164.

We demonstrate that recombinant viruses formed between a wild-type virus and a viral transgene can be isolated from transgenic plants under conditions of moderate to weak selection pressure. We inoculated cauliflower mosaic virus (CaMV) strain W260 to transgenic Nicotiana bigelovii plants that expressed a copy of CaMV gene VI derived from CaMV strain D4, a gene that determines systemic infection of solanaceous species, including N. bigelovii. Because W260 infects nontransformed N. bigelovii systemically, a recombinant virus formed between W260 and the D4 transgene would be expected to have little selective advantage over the wild-type W260 virus W260 was inoculated to approximately 100 plants each of nontransformed and transgenic N. bigelovii and it systemically infected nearly all of the plants. An analysis of viral DNA recovered from 23 transgenic plants infected with W260 revealed that 20 infections resulted from the systemic movement of the wild-type W260 virus, while a recombinant between W260 and the D4 transgene was detected in three of the infections. To determine the percentage of recovery of recombinant viruses under strong selection pressure, we inoculated approximately 100 nontransformed and 100 D4 gene VI transgenic plants with CaMV strain CM1841, a virus that is unable to infect nontransformed N. bigelovii. CM1841 infected 36% of the transgenic plants systemically, but none of the nontransformed controls. An analysis of 24 infected plants showed that a recombination event occurred in every plant, demonstrating that under strong selection conditions, the recovery of CaMV recombinants from transgenic plants can be very high.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S004268229690464X

A estrutura física do promotor 35S CaMV – que possui sequências nucleotídicas palindrômicas[26] – tende a favorecer recombinações genéticas ilegítimas, potencialmente geradoras de modificações genômicas inesperadas.

Muller, A.; Kamisugi, Y.; Gruneberg, R.; Niedenhof, I.; Horold, R.; Meyer, P. 1999. Palindromic sequences and A+T-rich DNA elements promote illegitimate recombination in *Nicotiana tabacum*. *J. Mol. Biol.,* 291, 29-46.

Illegitimate recombination is the prevailing molecular mechanism for the integration of recombinant DNA into the genome of most eukaryotic systems and the generation of deletions by intrachromosomal recombination. We developed a selectable marker system to screen for intrachromosomal illegitimate recombination events in order to assess the sequence and structure-specific requirements for illegitimate recombination in tobacco. In 12 illegitimate recombination

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[26] Trata-se de filamentos de DNA de polaridade inversa, porém com a mesma sequência de nucleotídeos. Considere como exemplo as sequências palindrômicas 5'-GAATTC-3' e 3'-CTTAAG-5'.

products analysed, we found that all deletion termini localise to sites of palindromic structures or to A+T-rich DNA elements. All deletion termini showed microhomologies of two to six nucleotides. In three plants, the recombination products contained filler-DNA or an inversion of an endogenous segment. Our data strongly suggest that illegitimate recombination in plants is mediated by a DNA synthesis-dependent process, and that this mechanism is promoted by DNA regions that can form palindromic structures or facilitate DNA unwinding.

http://www.ncbi.nlm.nih.gov/pubmed/10438604

Kohli, A.; Griffiths, S.; Palacios, N.; Twyman, R.; Vain, P.; Laurie, D.; Christou, P. 1999. Molecular characterization of transforming plasmid rearrangments in transgenic rice reveals a recombinaison hotspot in the CaMV 35S promoter and confirms the predominance of microhomologie mediated recombination. *The Plant Journal*, 17, 6, p. 591-601.

The characterization of plasmid-genomic DNA junctions following plant transformation has established links between DNA double-strand break repair (DSBR), illegitimate recombination and plasmid DNA integration. The limited information on plasmid-plasmid junctions in plants comes from the dicot species tobacco and Arabidopsis. We analyzed 12 representative transgenic rice lines, carrying a range of transforming plasmid rearrangements, which predominantly reflected microhomology mediated illegitimate recombination involving short complementary patches at the recombining ends. Direct end-ligation, in the absence of homology between the recombining molecules, occurred only rarely. Filler DNA was found at some of the junctions. Short, purine-rich tracts were present, either at the junction site or in the immediate flanking regions. Putative DNA topoisomerase I binding sites were clustered around the junctions. Although different regions of the transforming plasmid were involved in plasmid-plasmid recombination, we showed that a 19 bp palindromic sequence, including the TATA box of the CaMV 35S promoter, acted as a recombination hotspot. The purine-rich half of the palindromic sequence was specifically involved at the recombination junctions. This recombination hotspot is located within the 'highly recombinogenic' region of the full-length CaMV RNA that has been shown to promote viral recombination in dicot plants. Clustering of plasmid recombination events in this highly recombinogenic region, even in the absence of viral enzymes and other cis-acting elements proves that the plant cellular machinery alone is sufficient to recognize and act on these viral sequences. Our data also show the similarity between mechanisms underlying junction formation in dicot and monocot plants transformed using different procedures.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1046/j.1365-313X.1999.00399.x/pdf

REV – Ho, M.; Ryan, A.; Cummins, J. 2000. Hazards of transgenic plants containing the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease,* 12, 6-11.

Sem resumo.

Artigo completo disponível em http://www.i-sis.org.uk/pdf/CaMV_promoter_hazards_of_transgenic_plants.pdf

COM – Cummins, J.; Ho, M.; Ryan, A. 2000. Hazardous CaMV promoter? *Nature Biotechnology,* 18, 363.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/10748474

Podevin, N.; du Jardin, P. 2012. Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants.*GM Crops and Food*, 3 (4): 296-300.

Multiple variants of the Cauliflower mosaic virus 35S promoter (P35S) are used to drive the expression of transgenes in genetically modified plants, for both research purposes and commercial applications. The genetic organization of the densely packed genome of this virus results in sequence overlap between P35S and viral gene VI, encoding the multifunctional P6 protein. The present paper investigates whether introduction of P35S variants by genetic transformation is likely to result in the expression of functional domains of the P6 protein and in potential impacts in transgenic plants. A bioinformatic analysis was performed to assess the safety for human and animal health of putative translation products of gene VI overlapping P35S. No relevant similarity was identified between the putative peptides and known allergens and toxins, using different databases. From a literature study it became clear that long variants of the P35S do contain an open reading frame, when expressed, might result in unintended phenotypic changes. A flowchart is proposed to evaluate possible unintended effects in plant transformants, based on the DNA sequence actually introduced and on the plant phenotype, taking into account the known effects of ectopically expressed P6 domains in model plants.

http://www.ncbi.nlm.nih.gov/pubmed/22892689

Considerando que o P35S CaMv pode ser ativo em um grande conjunto de organismos vivos (e não apenas em plantas), deve-se considerar aspectos de biossegurança associados à possibilidade de esse promotor permitir a expressão de novos elementos de DNA – recombinados ou não – sob seu controle, uma vez ingerido, integrado por organismos competentes via THG[27] (bactérias simbiontes do ser humano, por exemplo) ou disseminado no meio ambiente.

Ballas, N.; Broido, S.; Soreq, H.; Loyter, A. 1989. Efficient functioning of plant promoters and poly(A) sites in *Xenopus* oocytes. *Nucleic Acids Res.,* 17: 7891–7903.

Mature Xenopus oocytes were challenged with DNA constructs including plant regulatory elements, namely, the Cauliflower mosaic virus (CaMV) 35S promoter as well as the nopaline synthase (NOS) promoter and polyadenylation signal. The bacterial chloramphenicol acetyl transferase (CAT) was used as a reporter gene. When microinjected into these cells, the plant-derived DNA constructs effectively promoted CAT synthesis in a manner dependent on the presence of the plant promoters and probably also on the polyadenylation signals. Structural studies revealed that the supercoiled structures of the above DNA plasmids were much more active in supporting CAT synthesis in microinjected oocytes than their linear forms, with clear correlation between efficient gene expression and DNA topology. In contrast, the linear forms of these plasmids were considerably more active than the supercoiled ones in transfected plant protoplasts. These findings demonstrate, for the first time, the activity of regulatory elements from plant genes in Xenopus oocytes and shed new light on the specific rules applicable for gene expression in plant and animal cells.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC334895/

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[27] Ver item 4.2 da Parte 4, onde são apresentados artigos que tratam de riscos associados a transferências horizontais envolvendo o promotor 35S do CaMV, em células de mamíferos.

Gmunder, H.; Kohli, J. 1989. Cauliflower mosaic virus promoters direct efficient expression of a bacterial G418 resistance gene in Schizosaccharomyces pombe. *Mol. Gen. Genet.*, 220: 95-101.

A system is presented for transformation of the fission yeast Schizosaccharomyces pombe to resistance against the antibiotic G418. The bacterial resistance gene of the transposon Tn5 is expressed under the control of promoters and transcription terminators from cauliflower mosaic virus (CaMV). The promoter of the S. pombe alcohol dehydrogenase gene has also been used. Transformants can be selected directly on medium containing G418 (up to 1 mg/ml) due to inactivation of G418 by the Tn5 gene product, the aminoglycoside 3'-phosphotransferase (II). The plant viral promoter 35S confers higher resistance to G418 than the 19S promoter. This corresponds to the relative strengths of these promoters in plant cells. The strong plant promoter 35S yields resistance comparable to that obtained with the strong S. pombe promoter from the alcohol dehydrogenase gene. The constructions with the two plant promoters have been used on multicopy shuttle plasmids that replicate autonomously in S. pombe and Escherichia coli. In addition the 35S and the 19S constructions have been inserted into the S. pombe genome where they confer G418 resistance as single copy genes. Since vector sequences are excluded in this case, all the necessary signals for expression of G418 resistance are contained within the DNA fragments containing the plant promoters, the resistance gene and the plant terminators. This transformation system is independent of S. pombe mutants. It may be useful for the transformation of other lower eukaryotes. The activity of the CaMV promoters in S. pombe may be exploited for the expression of plant genes in fission yeast.

http://www.ncbi.nlm.nih.gov/pubmed/2558289

Hirt, H.; Kogl, M.; Murbacher, T.; Heberlebors, E. 1990. Evolutionary conservation of transcriptional machinery between yeast and plants as shown by the efficient expression from the CaMV 35S promoter and 35S terminator. *Curr. Genet.,* 17: 473–479.

Complementation of fission yeast mutants by plant genomic libraries could be a promising method for the isolation of novel plant genes. One important prerequisite is the functioning of plant promoters and terminators in Schizosaccharomyces pombe and Saccharomyces cerevisiae. Therefore, we studied the expression of the bacterial beta-glucuronidase (GUS) reporter gene under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter and 35S terminator. We show here that S. pombe initiates transcription at exactly the same start site as was reported for tobacco. The 35S CaMV terminator is appropriately recognized leading to a polyadenylated mRNA of the same size as obtained in plant cells transformed with the same construct. Furthermore, the GUS-mRNA is translated into fully functional GUS protein, as determined by an enzymatic assay. Interestingly, expression of the 35S promoter in the budding yeast S. cerevisiae was found to be only moderate and about hundredfold lower than in S. pombe. To investigate whether different transcript stabilities are responsible for this enormous expression difference in the two yeasts, the 35S promoter was substituted by the ADH (alcohol dehydrogenase) promoter from fission yeast. In contrast to the differential expression pattern of the 35S promoter, the ADH promoter resulted in equally high expression rates in both fission and budding yeast, comparable to the 35S promoter in S. pombe. Since the copy number of the 35S-GUS constructs differs only by a factor of two in the two yeasts, it appears that differential recognition of the 35S promoter is responsible for the different transcription rates.

http://www.ncbi.nlm.nih.gov/pubmed/2202523

Assaad, F.; Signer, E. 1990. Cauliflower mosaic-virus p35S promoter activity in *Escherichia-coli. Molecular and General Genetics,* 223(3): 517-520.

We present evidence that the cauliflower mosaic virus promoter P35S can direct expression of the bacterial neomycin phosphotransferase II (NPTII) gene in Escherichia coli. Transcription is initiated at several sites, the major one being located approximately 315 bases upstream of the plant start site. The nucleotide sequence directly preceding this start site is strongly homologous to the prokaryotic promoter consensus sequence. Thus constructs designed for introduction into plants can be expressed in E. coli.

http://www.ncbi.nlm.nih.gov/pubmed/2176717

Burke, C.; Yu X.; Marchitelli, L.; Davis, E.; Ackerman, S. 1990. Transcription Factor IIA of wheat and human function similarly with plant and animal viral promoters. *Nucleic Acid Research,* 18(12):3611-3620.

Eucaryotic transcription initiation by RNA polymerase II involves protein:DNA interactions during the formation of a transcription complex. In addition to RNA polymerase II there are at least five other general transcription factors necessary for initiation with the adenovirus major late promoter. One of these, TFIIA, is involved in the earliest events during transcription complex assembly. We have purified TFIIA from wheat germ and characterized it in an in vitro transcription system. Wheat TFIIA is a single polypeptide of Mr approximately 35 kd which functionally replaces human (HeLa) TFIIA to form a wheat/HeLa transcription system. [This polypeptide can be eluted from a SDS-polyacrylamide gel, refolded to a native conformation, and will function as wheat TFIIA in the heterologous system.] The heterologous system requires a lower optimal incubation temperature than the HeLa system. Biochemical characterization, using the adenovirus major late promoter, indicates that transcription reaction parameters for both wheat and HeLa TFIIA are similar but the kinetics of transcription for both TFIIAs are somewhat dissimilar. A plant viral promoter, the cauliflower mosaic virus 35S promoter, accurately and efficiently directs in vitro transcription in both the wheat/HeLa and HeLa systems with identical transcription kinetics. We conclude that TFIIA function has been conserved during evolution.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC331017/

Pobjecky, N.; Rosenberg, G.; Dintergottlieb, G.; Kaufer, N. 1990. Expression of the betaglucuronidase gene under the control of the CaMV-35S promoter in Schizosaccharomyces-pombe. *Molecular & General Genetics,* 220 (2): 314-316.

We have transformed Schizosaccharomyces pombe with the beta-glucuronidase (GUS) gene from Escherichia coli under the control of the plant cauliflower mosaic virus (CaMV) 35S promoter element. Efficient expression of GUS enzyme was observed. Moreover, transcription initiated at a unique site identical to that used in plant cells.

http://www.ncbi.nlm.nih.gov/pubmed/2325625

Ruth, J.; Hirt, H.; Schweyen, R. 1992. The cauliflower mosaic virus 35S promoter is regulated by cAMP in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.,* 235:365–372.

The cauliflower mosaic virus 35S promoter confers strong gene expression in plants, animals and fission yeast, but not in budding yeast. On investigating this paradox, we found that in budding yeast the promoter acts through two domains. Whereas the upstream domain acts as a silencer, the downstream domain couples expression to the nutritional state of the cells via the RAS/cAMP pathway. Point mutations indicate that two boxes with similarity to the cAMP regulated element (CRE) of mammalian cells mediate this response. Gel retardation assays show that, in both yeast and plant protein extracts, factors bind to this promoter element. Therefore, transcriptional activation appears to be highly conserved at the level of transcription factors and specific DNA target elements in eukaryotes. This offers new ways to investigate gene regulation mechanisms of higher eukaryotes, which are not as amenable to genetic analysis as yeast.

http://www.ncbi.nlm.nih.gov/pubmed/1334531

Lewin, A.; Jacob, D.; Freytag, B.; Appel, B. 1998. Gene expression in bacteria directed by plant-specific regulatory sequences. *Transgenic Research*, 7:403-411.

The regulation of gene expression represents a specific process which has different structural and functional requirements in different groups of organisms. It is thus assumed that regulatory sequences of eucaryotes cannot be recognized in procaryotes. This assumption is of interest for risk assessments of the environmental impact of deliberate release experiments with genetically modified organisms. In order to analyse the extent of heterologous gene expression caused by the transfer of plant-specific regulatory sequences into bacteria, we constructed fusions between plant-specific regulatory sequences and the coding regions of the luxAB genes for the luciferase of the bioluminescent bacterium Vibrio harveyi, transferred the fusions into different bacterial species and measured the luminescence to quantify the expression of the luciferase genes. The regulatory sequences investigated included (a) the 35S promoter of the Cauliflower mosaic virus, (b) the B33 promoter of a class I patatin gene of potatoes, (c) the promoter of the ST-LS1 gene of potatoes and (d) the promoter of the rolC gene of Agrobacterium rhizogenes. We could show that in addition to the 35S promoter, which has already been described as being recognized in Escherichia coli, the sequences containing the B33 and the ST-LS1 promoters are recognized in bacteria. Luciferase gene expression promoted by the sequence with the ST-LS1 promoter could be observed in E. coli, Yersinia enterocolitica and Agrobacterium tumefaciens. Comparison of the luminescence caused by fusions between luxAB and different promoters on the chromosome and on an endogenous plasmid of Y. enterocolitica demonstrated that the level of the heterologous gene expression caused by the fragment with the ST-LS1 promoter was within the range of gene expression levels caused by endogenous promoters of Y. enterocolitica.

http://link.springer.com/article/10.1023/A%3A1008876826415

Ryabova, L.; Hohn, T. 2000. Ribosome shunting in cauliflower mosaic virus 35S RNA leader is a special case of reinitiation of translation functioning in plant and animal systems. *Genes Dev.,* 14: 817-829.

The shunt model predicts that small ORFs (sORFs) within the cauliflower mosaic virus (CaMV) 35S RNA leader and downstream ORF VII are translated by different mechanisms, that is, scanning-reinitiation and shunting, respectively. Wheat germ extract (WGE) and rabbit reticulocyte lysate (RRL) in vitro translation systems were used to discriminate between these two processes and to study the mechanism of ribosomal shunt. In both systems, expression downstream of the leader occurred via ribosomal shunt under the control of a stable stem and a small ORF preceding it. Shunting ribosomes were also able to initiate quite efficiently at non-AUG start codons just downstream of the shunt landing site in WGE but not in RRL. The short sORF MAGDIS from the mammalian AdoMetDC RNA, which conditionally suppresses reinitiation at a downstream ORF, prevented shunting if placed at the position of sORF A, the 5'-proximal ORF of the CaMV leader. We have demonstrated directly that sORF A is translated and that proper termination of translation at the 5'-proximal ORF is absolutely required for both shunting and linear ribosome migration. These findings strongly indicate that shunting is a special case of reinitiation.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC316492/

COM – Ho, M.; Ryan, A.; Cummins, J. 2000. CaMv 35S Promoter Fragmentation Hotspot Confirmed, and it is Active in Animals. *Microbial Ecology in Health and Disease,* 12, 189.

Sem resumo.

Artigo completo disponível em http://www.i-sis.org.uk/pdf/CAMV_35s_promo_hotspot_confirmed.pdf

Jacob, D.; Lewin, A.; Meister, B.; Appel, B. 2002. Plant-specific promoter sequences carry elements that are recognised by the eubacterial transcription machinery. *Trans. Research,* 11, 291-303.

During evolution the promoter elements from prokaryotes and eukaryotes have developed differently with regard to their sequence and structure, implying that in general a transfer of eukaryotic promoter sequences into prokaryotes will not cause an efficient gene expression. However, there have been reports on the functionality of the 35S promoter from cauliflower mosaic virus (CaMV) in bacteria. We therefore decided to experimentally investigate the capability of plant promoter sequences to direct gene expression in various bacteria. Accordingly, we tested ten different plant-specific promoters from Solanum tuberosum, Nicotiana tabacum, CaMV, Agrobacterium tumefaciens, and A. rhizogenes for their ability to initiate transcription in five different eubacterial species (Escherichia coli, Yersinia enterocolitica, A. tumefaciens, Pseudomonas putida, and Acinetobacter sp. BD413). To monitor the strength of the plant-specific promoters in bacteria we created fusions between these promoters and the coding region of the luciferase genes from Vibrio harveyi and measured the luminescence in the bacteria. Heterologous gene expression was observed in 50% of the combinations analysed. We then mapped the transcription start site caused by one of the plant-specific promoters, the ST-LS1 promoter from S. tuberosum, in these bacterial species. The location of the mapped transcription start site indicated that the sequences of the plant promoter themselves were recognised by the bacterial transcription apparatus. The recognition of plant-specific promoter sequences by the bacterial RNA polymerase was further confirmed by site-directed mutagenesis of the ST-LS1 promoter and the analysis of the effects of the mutations on the strength of gene expression in E. coli. Using these mutants in our reporter assays we could localise the sequences of the ST-LS1 promoter serving as -10 region in E. coli. The results of our study show that promoter sequences are much less specific than is generally assumed. This is of great importance for our knowledge about the evolution of gene expression systems and for the construction of optimised expression vectors.

http://www.ncbi.nlm.nih.gov/pubmed/12113461

Vlasak, J.; Smahel, M.; Pavlik, A.; Pavingerova, D.; Briza, J. 2003. Comparison of hCMV immediate early and CaMV 35S promoters in both plant and human cells. *J. Biotechnol.*, 103, 197-202.

Cauliflower mosaic virus 35S promoter, widely used in transgenic crop plants, is known to be recognized in widely differing kinds of cells. Its activity in human cells may have impact on the risk assessment for the environmental release of genetically modified plants. In this study, transient expression of several constructs containing beta-glucuronidase (GUS) gene driven by cauliflower mosaic virus 35S promoter or by immediate early promoter of human cytomegalovirus (pCMV) was tested in both potato leaf protoplasts and cultured human cells. The results showed very low but measurable activity of 35S promoter in human 293T-cells (0.01% of that revealed when using pCMV) and in 293 cells that do not produce SV40 T antigen this activity was even lower. On the other hand, in potato protoplasts, pCMV displayed nearly 1% activity seen with p35S.

http://www.ncbi.nlm.nih.gov/pubmed/12890606

Tepfer, M. ; Gaubert, S. ; Leroux-Coyau, M. ; Prince, S. ; Houdebine, L. 2004. Transient expression in mammalian cells of transgenes transcribed from the Cauliflower mosaic virus 35S promoter. *Environ. Biosafety Res.,* 3, 91-97.

Gene constructs containing the Cauliflower mosaic virus (CaMV) 35S promoter and a sequence coding either for a green fluorescent protein (GFP) or for firefly luciferase were transfected into Chinese hamster ovary (CHO) cells. Both reporter genes were expressed to significant levels. The 35S promoter was 40 times less active than the human eF1 alpha promoter, which is known to be one of the most potent promoters in mammalian cells. The 35S promoter must therefore be considered to be a promoter of significant potency in mammalian cells. RT-PCR analysis suggested that transcription initiation in CHO cells occurred between the TATA box and the transcription start site of the 35S promoter that function in plant cells. Further analysis by 5'RACE confirmed that transcription was initiated in CHO cells at different sites located essentially between the TATA box and the plant transcription start site, showing that 35S promoter activity in animal cells is due to the presence of promoter elements that are functional in mammalian cells, but that are not those used in plants. The data reported here raise the possibility that genes controlled by the 35S promoter, which is commonly used in transgenic plants, have the potential for expression in animal cells.

http://www.ncbi.nlm.nih.gov/pubmed/15612506

Myhre, M.; Fenton, K.; Eggert, J.; Nielsen, K.; Traavik, T. 2006. The 35S CaMV plant virus promoter is active in human enterocyte-like cells. *Eur Food Res Technol,* 222: 185–193.

The 35S cauliflower mosaic virus (CaMV) promoter is commonly used to drive transgene expression in the genetically engineered (GE) crop plants that have been commercialized so far. Whether, and how far, the 35S promoter might be active in mammalian cells has been scientifically unsettled and controversial. Very recently it was established that the 35S promoter is transcriptionally active following transient reporter gene transfections in continuous cell lines of human [J Biotechnol 103:197–202, 2003] and hamster ovary [Environ Biosafety Res 3:41–47, 2004] fibroblasts. The initial exposure of a human organism to DNA from GE food takes place in the gastrointestinal tract (GIT). Hence, we have now investigated the promoter capacity of 35S in human enterocyte-like cells. We constructed expression vectors with 35S promoter inserted in front of two reporter genes encoding firefly luciferase and green fluorescent protein (GFP), respectively, and performed transient transfection experiments in the human enterocyte-like cell line Caco-2. It was demonstrated that the 35S CaMV promoter was able to drive the expression of both reporter genes to significant levels, although the protein expression levels might seem modest compared to those obtained with the strong promoters derived from human cytomegalo virus (hCMV) and simian virus 40 (SV40). Furthermore, computer-based searches of the 35S CaMV DNA sequence for putative mammalian transcription factor binding motifs gave a high number of hits. Some of the identified motifs indicate that transcriptional activation by the 35S CaMV promoter may be stronger in other human and animal cell types than in those investigated so far.

Artigo completo disponível em http://www.mma.gov.br/estruturas/biosseguranca/_arquivos/71_28112008024750.pdf

3.3 Riscos associados ao uso de *Agrobacterium tumefaciens* em plantas transgênicas

A Agrobacterium tumefaciens é naturalmente competente para realização de transferência horizontal de genes. Por essa razão, a bactéria tem sido usada para efetuar inserção de genes desejados em plantas cultivadas, transgênicas ou não. Recentemente observou-se que ela também é capaz de transferir material genético entre determinadas espécies de fungos e em células humanas, em condições seminaturais. As implicações são relevantes e sugerem que o uso dessa bactéria para a transformação de plantas pode vir a resultar na inserção de genes não desejados, próprios ao genoma de *A. tumefaciens*, tais como aqueles responsáveis pelos tumores nas plantas infectadas. A possibilidade de o DNA recombinante ser exsudado pelas raízes das plantas transformadas e transferido para cepas de *A. tumefaciens* naturais do solo (ou para outras bactérias competentes) – evento favorecido pelas homologias das sequências genômicas – sugere riscos potenciais importantes e irreversíveis, associados à disseminação de transgenes no meio ambiente.

Nesse sentido, o uso de *A. tumefaciens* na transformação de PGM amplia fatores de risco associados a THG entre organismos presentes em áreas cultivadas com aquelas plantas transgênicas, organismos que as consomem[28] ou que com elas interagem.

Knight, C.; Bailey, A.; Foster, G. 2010. Investigating Agrobacterium-mediated transformation of *Verticillium* albo-atrum on plant surfaces. *PLOS ONE*, 5(10): 13684.

Background: *Agrobacterium tumefaciens* has long been known to transform plant tissue in nature as part of its infection process. This natural mechanism has been utilized over the last few decades in laboratories world wide to genetically manipulate many species of plants. More recently this technology has been successfully applied to non-plant organisms in the laboratory, including fungi, where the plant wound hormone acetosyringone, an inducer of transformation, is supplied exogenously. In the natural environment it is possible that *Agrobacterium* and fungi may encounter each other at plant wound sites, where acetosyringone would be present, raising the possibility of natural gene transfer from bacterium to fungus.
Methodology/Principal Findings: We investigate this hypothesis through the development of experiments designed to replicate such a situation at a plant wound site. *A. tumefaciens* harbouring the plasmid pCAMDsRed was co-cultivated with the common plant pathogenic fungus *Verticillium albo-atrum* on a range of wounded plant tissues. Fungal transformants were obtained from co-cultivation on a range of plant tissue types, demonstrating that plant tissue provides sufficient *vir* gene inducers to allow *A. tumefaciens* to transform fungi *in planta*.
Conclusions/Significance: This work raises interesting questions about whether *A. tumefaciens* may be able to transform organisms other than plants in nature, or indeed should be considered during GM risk assessments, with further investigations required to determine whether this phenomenon has already occurred in nature.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0013684

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[28] Riscos associados à THG são tratados nos itens 3.3 e 4.2 das Partes 3 e 4, respectivamente.

Kunik, T.; Tzfira, T.; Kapulnik, Y.; Gafni, Y.; Dingwall, C.; Citovsky, V. 2001. Genetic transformation of HeLa cells by Agrobacterium. *Proc Natl Acad Sci USA*, 98(4): 1871-1876.

Agrobacterium tumefaciens is a soil phytopathogen that elicits neoplastic growths on the host plant species. In nature, however, *Agrobacterium* also may encounter organisms belonging to other kingdoms such as insects and animals that feed on the infected plants. Can *Agrobacterium*, then, also infect animal cells? Here, we report that *Agrobacterium* attaches to and genetically transforms several types of human cells. In stably transformed HeLa cells, the integration event occurred at the right border of the tumor-inducing plasmid's transferred-DNA (T-DNA), suggesting bona fide T-DNA transfer and lending support to the notion that *Agrobacterium* transforms human cells by a mechanism similar to that which it uses for transformation of plants cells. Collectively, our results suggest that *Agrobacterium* can transport its T-DNA to human cells and integrate it into their genome.

Artigo completo disponível em http://www.pnas.org/content/98/4/1871.full

Barrett, C.; Cobb, E.; McNicol, R.; Lyon, G. 1997. A risk assessment study of plant genetic transformation using Agrobacterium and implications for analysis of transgenic plants. *Plant Cell, Tissue and Organ Culture*, 47: 135–144.

Agrobacterium transformation systems for *Brassica, Solanum* and *Rubus*, using carbenicillin, cefotaxime and ticaracillin respectively to eliminate contamination, were examined for the presence of residual *Agrobacterium*. The results indicated that none of the antibiotics in question, succeeded in eliminating *Agrobacterium* and the contamination levels increased in explants from 12 to 16 weeks to such an extent that *Solanum* cultures senesced and died. This may be due to the fact that four times the Minimum bactericidal concentration values (concentration to be used for elimination of contaminants in culture), for the three antibiotics, were higher than the concentrations employed in the culture medium. Contamination in shoot material decreased over 16 to 24 weeks possibly due to bacteriostatis and the use only of the apical node for further culture. The presence of the binary vector was also noted under non-selective conditions, even up to 6 months after transformation, where approx. 50% of contaminated material still harboured bacterial cells with the binary vector at levels of approx. 10_7 Colony forming units per gram.

http://link.springer.com/article/10.1007/BF02318949

Ulker, B.; Li, Y.; Rosso, M.; Logemann, E.; Somssich, I.; Weisshaar, B. 2008. T-DNA–mediated transfer of Agrobacterium tumefaciens chromosomal DNA into plants. *Nature Biotechnology*, volume 26, number 9.

Besides the well-documented integration of DNA flanked by the transfer DNA borders, occasional insertion of fragments from the tumor-inducing plasmid into plant genomes has also been reported during Agrobacterium tumefaciens-mediated transformation. We demonstrate that large (up to approximately 18 kb) gene-bearing fragments of Agrobacterium chromosomal DNA (AchrDNA) can be integrated into Arabidopsis thaliana genomic DNA during transformation. One in every 250 transgenic plants may carry AchrDNA fragments. This has implications for horizontal gene transfer and indicates a need for greater scrutiny of transgenic plants for undesired bacterial DNA.

http://www.ncbi.nlm.nih.gov/pubmed/18758448

Parte 2

Questões agronômicas relativas ao cultivo de plantas transgênicas

1 Produtividade, custo de produção e uso de agrotóxicos em lavouras de plantas transgênicas comerciais

Apesar de essa obra focalizar aspectos biológicos relacionados a riscos e incertezas associados ao uso comercial de plantas transgênicas, faz-se necessário abordar algumas questões relativas a seus desdobramentos socioeconômicos.

Aqui são apresentados estudos que questionam suposições reiteradas em campanhas de marketing e endossadas por estudos de duvidosa qualidade. Essas campanhas informam maior produtividade para plantas que foram transformadas visando apenas suportar banhos de herbicidas, ou redução nos custos de produção de lavouras cultivadas com sementes que custam quatro vezes mais, ou mesmo diminuição do uso de agrotóxicos, quando se percebe crescimento exponencial de suas vendas. Trata-se de discussão importante porque tais afirmativas continuam sendo repetidas, embora contrariadas pela realidade, e ainda assumem peso importante em tomadas de decisões que contribuem diretamente para autorizações de cultivo e comercialização de plantas transgênicas.

Com esse objetivo, no primeiro item deste capítulo apresentamos alguns estudos que contrariam argumentos produzidos pela indústria das biotecnologias ou a seu serviço (frequentemente reiterados por determinados autores em algumas publicações científicas). Nesse caso, o objetivo é destacar que também nesses pontos a controvérsia é intensa e as divergências estão longe de ser sanadas.

São muito variadas as condições que permitem oscilações nos indicadores mais relevantes para as campanhas promocionais dos transgênicos. Fundamentalmente, as respostas observadas em cada caso e para cada parâmetro dependerão de interações entre as particularidades do genoma e as condições ambientais, sendo óbvio o fato de que, na ausência de alterações que impactem diretamente fatores de construção da produtividade (absorção e metabolização de água e nutrientes, peso e número de grãos, utilização da energia solar, etc.), qualquer resultado nesse sentido será fruto de fatores aleatórios (de ordem genética, ambiental ou agronômica).

Assim, uma vez que as gerações de plantas transgênicas disponíveis no mercado não sofreram alterações que permitam ganhos de produtividade, e levando em conta a frequência com que são observadas quedas de rendimento e/ou maior uso de agrotóxicos, e, consequentemente, menor benefício econômico para o produtor (em especial no caso dos pequenos produtores e agricultores familiares que originalmente dispunham de sementes próprias), torna-se importante examinar estudos que apontam razões técnicas que permitem duvidar de mitos associados aos benefícios dessas culturas.

Elmore, R.; Roeth, F.; Nelson, L.; Shapiro, C.; Klein, R.; Knezevic, S.; Martin, A. 2001. Glyphosate resistant soyabean cultivar yields compared with sister lines. *Agronomy Journal*, 93, 408-412.

Herbicide-resistant crops like glyphosate resistant (GR) soybean [Glycine max (L.) Merr.] are gaining acceptance in U.S. cropping systems. Comparisons from cultivar performance trials suggest a yield suppression may exist with GR soybean. Yield suppressions may result from either cultivar genetic differentials, the GR gene/gene insertion process, or glyphosate. Grain yield of GR is probably not affected by glyphosate. Yield suppression due to the GR gene or its insertion process (GR effect) has not been reported. We conducted a field experiment at four Nebraska locations in 2 yr to evaluate the GR effect on soybean yield. Five backcross-derived pairs of GR and non- GR soybean sister lines were compared along with three high-yield, nonherbicide-resistant cultivars and five other herbicide-resistant cultivars. Glyphosate resistant sister lines yielded 5% (200 kg ha21) less than the non-GR sisters (GR effect). Seed weight of the non-GR sisters was greater than that of the GR sisters (in 1999) and the non-GR sister lines were 20 mm shorter than the GR sisters. Other variables monitored were similar between the two cultivar groups. The high-yield, nonherbicide-resistant cultivars included for comparison - yielded 5% more than the non-GR sisters and 10% more than the GR sisters.

Artigo completo disponível em http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1028&context=agronomyfacpub

Men, X.; Ge, F.; Edwards, C.; Yardim, E. 2004. Influence of Pesticide Applications on Pest and Predatory Arthropods Associated with Transgenic Bt Cotton and Nontransgenic Cotton Plants. *Phytoparasitica,* 32(3):246-254.

The effects of pesticide applications on pests (aphids and acarid mites) and predators (ladybeetles and spiders) were investigated in transgenic *Bt* cotton and nontransgenic cotton agroecosystems in 1999, 2000 and 2001. Transgenic cotton did not cause changes in populations of acarids and did not reduce numbers of predators considerably; its effects on aphids were inconsistent. Although insecticides were not applied against the main pest . cotton bollworm . on transgenic cotton, the total number of insecticide applications in 3 years was no less than the total applied on nontransgenic cotton, because additional applications were required against sucking pests on transgenic *Bt* cotton. Pesticide applications decreased numbers of aphids, acarids and predatory spiders significantly on both transgenic and nontransgenic cottons. The results suggest that the use of *Bt* cotton should be evaluated carefully in China.

Artigo completo disponível em http://www.biosci.ohio-state.edu/~soilecol/Full%20articles/Ecology%20and%20Ecotoxicology/2004/Influence%20of%20pesticide%20applications.pdf

Ma, B.; Subedi, K. 2005. Development, yield, grain moisture and nitrogen uptake of Bt corn hybrids and their conventional near-isolines. *Field Crops Research*, 93: 199-211.

There are concerns over the economic benefits of corn (Zea mays L.) hybrids with the Bt trait transferred from Bacillus thuringiensis. A field experiment including three to seven pairs of commercial hybrids and their transgenic Bt near-isolines were grown side-by-side for three consecutive years in Ottawa, Canada (458170N, 758450W; 93 m above sea level) to determine (i) which hybrid had the highest yielding potential, (ii) if there was a differential response of Bt and non-Bt hybrids to N application, and (iii) under natural infestation of European corn borer (ECB), whether there was a yield advantage of Bt over non-Bt hybrids to justify their cost.We found that some of the Bt hybrids took 2–3 additional days to reach silking and maturity, and produced a similar or up to 12% lower grain yields with 3–5% higher grain moisture at maturity, in comparison with their non-Bt counterpart. Although N application increased grain yield and N uptake in 2 of the 3 years, there was no N-by-hybrid interaction on yield or other agronomic traits. Most Bt hybrids had similar to or lower total N content in grain with higher N in stover than their respective non-Bt near-isolines. Under extreme weather conditions (e.g. cool air temperature at planting and severe drought during the development), some of the hybrids (both Bt and non-Bt) required up to 400 additional crop heat units (CHU) to reach physiological maturity than indicated by the supplying companies. Our data suggest that within the same maturity group, it was the superior hybrids (non-Bt trait) that led to the greatest N accumulation, and the highest grain yield. Under the conditions tested, there was no yield advantage of Bt hybrids in comparison with their conventional counterparts when stalk lodging and breakage of the non-Bt counterpart by ECB was low to moderate.

Artigo completo disponível em http://www.saveourseeds.org/downloads/Btmaize_inferior_yield.pdf

Hofs, J.; Fok, M.; Vaissayre, M. 2006. Impact of Bt cotton adoption on pesticide use by smallholders: A 2-year survey in Makhatini Flats (South Africa). *Crop Protection*, 25(9): 984–988.

This paper explores insecticide use in fields cropped with conventional or Bt cotton varieties in a smallholder farming area (Makhathini Flats, KwaZulu Natal, South Africa). The study was carried out during the 2002-2003 and 2003-2004 growing seasons as part of a broader survey based on daily monitoring of a sample of smallholdings. The adoption of Bt cotton led to a decrease in pyrethroid use, but the level of insect resistance of this cultivar was not sufficient to completely drop this pesticide from the spraying programme. On the other hand, organophosphates were still being applied in substantial amounts, thus raising questions as to the impact of Bt cotton adoption on farmers' health. The overall economic results obtained with Bt cotton were slightly positive despite the low cotton yields obtained in the Flats during our survey. Bt cotton adoption did lead to labour savings, but the extent of this gain was not as high as expected. In conclusion, cropping Bt cotton in Makhathini Flats did not generate sufficient income to expect a tangible and sustainable socioeconomic improvement due to the way the crop is currently managed. Adoption of an innovation like Bt cotton seems to pay only in an agro-system with a sufficient level of intensification.

http://publications.cirad.fr/une_notice.php?dk=533741

Gordon, B. 2007. Manganese nutrition of glyphosate resistant and conventional soybeans. *Better Crops,* 91 (4): 12-13.

This study was conducted to determine if glyphosate-resistant (GR) soybeans respond differently to Mn fertilizer than conventional soybean varieties in an irrigated high-yield environment, and if so to develop fertilization strategies that will prevent or correct deficiencies. Yield of the GR variety was less than the conventional variety without Mn fertilizer. However, Mn application (banded at planting) to the GR variety closed the yield gap. The conventional soybean variety was not responsive to Mn fertilization. Conversely, yield was reduced at the highest rate of Mn. A second phase of the study showed that a combination of Mn applied as starter and foliar application provided maximum yield response.

Artigo completo disponível em http://www.ipni.net/ppiweb/bcrops.nsf/$webindex/70abdb50a75463f085257394001b157f/$file/07-4p12.pdf

Swaminathan, M.; Rawal, V. 2011. Are there Benefits from the Cultivation of Bt cotton? *Review of Agrarian Studies,* Vol 1(1).

This note examines costs and returns from the cultivation of different types of cotton in a rainfed village in the Vidarbha region of Maharashtra, India. While the pros and cons of GM cotton are extensively debated, there are only a few empirical studies on the economic performance of Bt cotton, particularly under rainfed conditions. The results from a detailed survey of farm business incomes show that Bt cotton was a clear leader in terms of production and gross value of output when grown as a stand-alone crop. However, on the fields of small and marginal farmers, where cotton was usually intercropped with sorghum (or other cereals and pulses), the relative income advantage of Bt cotton declined. Further, expenditure on chemical pesticides was higher for Bt cotton than for other varieties of cotton. Variability in production was also higher for Bt cotton than for other types of cotton.

Artigo completo disponível em http://ras.org.in/are_there_benefits_from_the_cultivation_of_bt_cotton_a_comment_based_on_data_from_a_vidarbha_village

Benbrook, C. 2012. Impacts of genetically engineered crops on pesticide use in the U.S. the first sixteen years. *Environmental Sciences Europe,* 24:24.

Background: Genetically engineered, herbicide-resistant and insect-resistant crops have been remarkable commercial successes in the United States. Few independent studies have calculated their impacts on pesticide use per hectare or overall pesticide use, or taken into account the impact of rapidly spreading glyphosate-resistant weeds. A model was developed to quantify by crop and year the impacts of six major transgenic pest-management traits on pesticide use in the U.S. over the 16-year period, 1996–2011: herbicide-resistant corn, soybeans, and cotton; Bacillus thuringiensis

(Bt) corn targeting the European corn borer; Bt corn for corn rootworms; and Bt cotton for Lepidopteron insects.
Results: Herbicide-resistant crop technology has led to a 239 million kilogram (527 million pound) increase in herbicide use in the United States between 1996 and 2011, while Bt crops have reduced insecticide applications by 56 million kilograms (123 million pounds). Overall, pesticide use increased by an estimated 183 million kgs (404 million pounds), or about 7%.
Conclusions: Contrary to often-repeated claims that today's genetically-engineered crops have, and are reducing pesticide use, the spread of glyphosate-resistant weeds in herbicide-resistant weed management systems has brought about substantial increases in the number and volume of herbicides applied. If new genetically engineered forms of corn and soybeans tolerant of 2,4-D are approved, the volume of 2,4-D sprayed could drive herbicide usage upward by another approximate 50%. The magnitude of increases in herbicide use on herbicide-resistant hectares has dwarfed the reduction in insecticide use on Bt crops over the past 16 years, and will continue to do so for the foreseeable future.

Artigo completo disponível em http://www.enveurope.com/content/24/1/24

Heinemann, J.; Massaro, M.; Coray, D.; Agapito-Tenfen, S.; Wen, J. 2013. Sustainability and innovation in staple crop production in the US Midwest. *International Journal of Agricultural Sustainability*, http://dx.doi.org/10.1080/14735903.2013.806408.

An agroecosystem is constrained by environmental possibility and social choices, mainly in the form of government policies. To be sustainable, an agroecosystem requires production systems that are resilient to natural stressors such as disease, pests, drought, wind and salinity, and to human constructed stressors such as economic cycles and trade barriers. The world is becoming increasingly reliant on concentrated exporting agroecosystems for staple crops, and vulnerable to national and local decisions that affect resilience of these production systems. We chronicle the history of the United States staple crop agroecosystem of the Midwest region to determine whether sustainability is part of its design, or could be a likely outcome of existing policies particularly on innovation and intellectual property. Relative to other food secure and exporting countries (e.g. Western Europe), the US agroecosystem is not exceptional in yields or conservative on environmental impact. This has not been a trade-off for sustainability, as annual fluctuations in maize yield alone dwarf the loss of caloric energy from extreme historic blights. We suggest strategies for innovation that are responsive to more stakeholders and build resilience into industrialized staple crop production.

Artigo completo disponível em http://sustainablepulse.com/wp-content/uploads/Jack.pdf

2 Baixa eficiência das plantas transgênicas no manejo de pragas e doenças

Poucos anos após a disponibilização em escala comercial de biotecnologias envolvendo plantas-inseticidas (Bt) e plantas com tolerância a herbicidas (TH), os produtores de plantas transgênicas passaram a enfrentar dificuldades no manejo e no controle de determinadas espécies de insetos considerados pragas e de plantas ruderais (erroneamente denominadas "invasoras, ou "daninhas"[29]). Efetivamente, a síntese continuada e massiva de proteínas Bt, assim como o uso sistemático e continuado dos mesmos princípios e ingredientes com atividades herbicidas, termina por gerar descomunal pressão seletiva sobre os organismos dos agroecossistemas envolvidos.

Ora, na diversidade intraespecífica de insetos e plantas ruderais, são encontrados indivíduos naturalmente resistentes às toxinas Bt e aos princípios ativos de herbicidas. Estes sobreviverão naqueles agrossistemas de plantas transgênicas, contrariamente à maioria dos outros organismos, naturalmente sensíveis às referidas tecnologias. Ao longo das gerações (e em alguns casos, na dependência da ecologia dos seres envolvidos, isso se dá em poucas safras agrícolas), os poucos indivíduos inicialmente selecionados por suas resistências geram progênies importantes a ponto de formar populações inteiras dotadas desse perfil genético evolutivamente vantajoso – no contexto transformado pela presença daquelas toxinas.

Dessa forma, a emergência e o desenvolvimento de populações resistentes aos pacotes tecnológicos associados a plantas GM do tipo BT e TH têm se conformado em espécie de condição geral. Observada em diversos locais do planeta, com ênfase para aquelas regiões onde os transgênicos se expandiram de maneira mais precoce e massiva, tal condição já alcança várias espécies, comprometendo a eficácia daquelas tecnologias. Os impactos socioeconômicos daí decorrentes são expressivos e agravados pelo fato de que o caráter de resistência, uma vez incorporado ao genoma das chamadas "pragas" e "plantas invasoras", se faz irreversível, tornando complexas as formas de controle e impedindo adoção de estratégias que eram eficazes anteriormente à expansão das tecnologias Bt e TH.

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[29] A expressão ruderal empregada ao longo desta publicação é no sentido proposto por Schneider (2007) (Schneider, A. A. A flora naturalizada no estado do Rio Grande do Sul, Brasil: herbáceas subespontâneas. Biociências, Porto Alegre, v. 15, nº 2, p. 257-268, jul. 2007) e diz respeito às espécies vegetais que se desenvolvem sem cultivo e sem cuidado humano, englobando tanto as espécies nativas (autóctones) quanto as naturalizadas. Ao contrário do termo "daninho", ruderal não tem juízo de valor e recusa a premissa falsa de que qualquer planta que não seja a cultura objeto seria prejudicial, o que não corresponde à verdade dos sistemas naturais que têm a diversidade como elemento inerente essencial à homeostase.

2.1 Desenvolvimento de populações de insetos resistentes às principais proteínas Bt

Mesmo antes do uso comercial em larga escala de plantas transgênicas do tipo Bt, a adaptação de populações de insetos às toxinas Cry gerava preocupações na comunidade científica. As hipóteses de rápida perda de eficácia toxicológica/agronômica dessas proteínas inseticidas apontavam necessidade de cautela em sua utilização, que deveria ser tópica, concentrada em focos de incidência de insetos e utilizada apenas diante de expectativas de danos econômicos. Com plantas que produzem permanentemente essas proteínas em todas suas células, implicando uso massivo independentemente da presença das pragas, seria de esperar rápida emergência de insetos resistentes. Esse fato tem sido observado em tempos que oscilam em torno dos cinco anos após o cultivo comercial, em larga escala, de milho, soja e algodão Bt, em diversos países e continentes.

REV – McGaughey, W.; Whalon, M. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. *Science,* 258: 1451-1455.

Bacillus thuringiensis (B.t.) δ-endotoxins provide an alternative to chemical insecticides for controlling many species of pest insects. Recent biotechnological developments offer the promise of even greater use of B.t. toxins in genetically transformed pest-resistant crops. However, the discovery that insects can adapt to these toxins raises concerns about the long-term usefulness of B.t. toxins. Several methods for managing the development of resistance to B.t. toxins have been suggested, but none of these approaches offer clear advantages in all situations.

http://www.ncbi.nlm.nih.gov/pubmed/17755107

COM – Kaiser, J. 1996. Pests Overwhelm Bt Cotton Crop. *Science*, vol. 273

Thousands of acres of cotton bioengineered to make its own insecticide have fallen victim in the southern United States to cotton bollworms, one of three pests that the crops were supposed to kill. The result has heightened the fears of environmental activists that the insects will eventually develop resistance to the toxin, known as Bt, and that fear has revived calls for tougher federal biosafety regulations. The reasons behind the disappointing results—involving one of the first large-scale plantings of a transgenic crop—also serve as a reminder to researchers that Mother Nature still has a few tricks up her sleeve.

http://www.sciencemag.org/content/273/5274/423.summary

2.1.1 Alto potencial de desenvolvimento de populações resistentes às toxinas Bt (observações em bioensaios)

Casos de resistência em populações de insetos obtidos em laboratórios são conhecidos desde mais de duas décadas. Estudos de bioensaios permitem avaliar o potencial de resistência de espécies com determinado perfil genético perante várias proteínas Bt. Sabe-se atualmente que tais populações podem se tornar insensíveis às toxinas em apenas uma ou duas dúzias de gerações – alguns anos para algumas espécies de insetos de ciclo biológico curto.

Ali, M.; Luttrell, R.; Young, S. 2006. Susceptibilities of *Helicoverpa zea* and *Heliothis virescens* (*Lepidoptera: Noctuidae*) Populations to Cry1Ac Insecticidal Protein. *J. Econ. Entomol.,* 99(1): 164-175.

Susceptibilities of bollworm, *Helicoverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (F.) to Cry1Ac were measured via a diet-incorporated assay with MPV II at the University of Arkansas during 2002–2004. Lethal concentration–mortality (LC_{50}) estimates of five laboratory, seven laboratory-cross, and 10 field populations of *H. virescens* varied 12-fold. Pooled susceptibilities of *H. virescens* across all laboratory and field populations varied five-fold. The LC_{50} estimates for *H. virescens* were higher than those reported by previous research before the introduction of transgenic crops. However, the ratio of susceptibility of laboratory and field populations was similar, suggesting no change in overall species susceptibility. Individual LC_{50} estimates of five laboratory, nine laboratory-cross, and 57 field populations of *H. zea* varied over 130-fold. Pooled susceptibilities across laboratory and field populations varied widely. Among the field populations, colonies from non-*Bacillus thuringiensis* (Bt) crops were generally more susceptible than those from Bt crops. Across the Bt crops expressing Cry protein, colonies from Bollgard (Monsanto Company) cotton had lower susceptibility to Cry1Ac than those from Bt corn and those from non-Bt crops.

http://www.ncbi.nlm.nih.gov/pubmed/16573337

Huang, F.; Leonard, B.; Wu, X. 2007. Resistance of sugarcane borer to *Bacillus thuringiensis* Cry1Ab toxin. *Entomologia Experimentalis et Applicata*,124: 117-123.

The sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), strain (F52-3-R) was developed from F_3 survivors of a single-pair mating on commercial Cry1Ab *Bacillus thuringiensis* (Bt) corn plants in the greenhouse. The susceptibility of a Bt-susceptible and the F52-3-R strain of *D. saccharalis* to trypsin-activated Cry1Ab toxin was determined in a laboratory bioassay. Neonatestage larvae were fed a meridic diet incorporating Cry1Ab toxin at a concentration range of 0.0625 to 32mg g^{-1}. Larval mortality, larval weight, and number of surviving larvae that did not gain significant weight (<0.1 mg per larva) were recorded on the 7th day after inoculation. The F52-3-R strain demonstrated a significant level of resistance to the activated Cry1Ab toxin. Larval mortality of the Bt-susceptible strain increased in response to higher concentrations of Cry1Ab toxin, exceeding 75% at 32mg g^{-1}, whereas mortality of the F52-3-R strain was below 8% across all Cry1Ab concentrations. Using a measure of practical mortality (larvae either died or gained no weight), the median lethal concentration (LC50) of the F52-3-R strain was 102-fold greater than that of the Bt-susceptible insects. Larval growth of both Bt-susceptible and F52-3-R strains was inhibited on Cry1Ab-treated diet, but the inhibition of the F52-3-R strain was significantly less than that of the Bt-susceptible insects. These results confirm that the survival of the F52-3-R strain on commercial Bt corn plants was related to Cry1Ab protein resistance and suggest that this strain may have considerable value in studying resistance management strategies for Bt corn.

http://onlinelibrary.wiley.com/doi/10.1111/j.1570-7458.2007.00560.x/abstract?deniedAccessCustomisedMessage=&userIsAuthenticated=false

Ali, M. ; Luttrell, R. 2007. Susceptibility of Bollworm and Tobacco Budworm (*Lepidoptera: Noctuidae*) to Cry2Ab2 Insecticidal Protein. *J. Econ. Entomol.,* 100(3): 921-931.

Susceptibilities of 82 bollworm, Helicoverpa zea (Boddie), and 44 tobacco budworm, Heliothis virescens (F.) (Lepidoptera: Noctuidae), populations to Cry2Ab2 protein were measured in diet incorporated assays at the University of Arkansas from 2002 to 2005. Resulting data were used to calculate overall (pooled data) estimates of species susceptibility for future benchmarks of resistance. Variabilities among populations also were studied by comparing regressions for individual populations and calculating mean susceptibilities for different subgroups of the colonies studied. Individual lethal concentration (LC50) estimates for nine laboratory, seven laboratory-cross, and 28 field populations of H. virescens varied up to 48-fold when adjusted for the response of the most susceptible laboratory colony studied. Mean susceptibilities of all laboratory, laboratory-cross, or field colonies varied only two-fold. When grouped by host plants, populations collected on tobacco, Nicotiana tabacum (L.), seemed to be less susceptible than those collected on other host plants. Individual LC50 values for 82 laboratory, laboratory-cross and field populations of H. zea varied up to 37-fold. Mean LC50 values of all laboratory, laboratory-cross, or field populations varied only three-fold. Susceptibilities of populations from Bollgard cotton were up to four-fold less than those from Bacillus thuringiensis corn, Zea mays L. Field populations collected during late season were generally less susceptible than those collected early in the season. Across the two species, H. zea was less sensitive to Cry2Ab2 than H. virescens. Both species seem to be less sensitive to Cry2Ab2 than to CrylAc.

http://www.ncbi.nlm.nih.gov/pubmed/17598557

Meihis, L.; Higdon, M.; Siegfried, B.; Miller, N.; Sappington, T.; Ellersieck, M.; Spencer, T.; Hibbard, B. 2008. Increased survival of western corn rootworm on transgenic corn within 3 generations of on-plant greenhouse selection. *Proc Natl Acad Sci USA*, 105(49): 19177-82.

To delay evolution of insect resistance to transgenic crops producing *Bacillus thuringiensis* (Bt) toxins, nearby "refuges" of host plants not producing Bt toxins are required in many regions. Such refuges are expected to be most effective in slowing resistance when the toxin concentration in Bt crops is high enough to kill all or nearly all insects heterozygous for resistance. However, Bt corn, *Zea mays*, introduced recently does not meet this "high-dose" criterion for control of western corn rootworm (WCR), *Diabrotica virgifera virgifera*. A greenhouse method of rearing WCR on transgenic corn expressing the Cry3Bb1 protein was used in which approximately 25% of previously unexposed larvae survived relative to isoline survival (compared to 1–4% in the field). After three generations of full larval rearing on Bt corn (Constant-exposure colony), WCR larval survival was equivalent on Bt corn and isoline corn in greenhouse trials, and the LC_{50} was 22-fold greater for the Constant-exposure colony than for the Control colony in diet bioassays with Cry3Bb1 protein on artificial diet. After six generations of greenhouse selection, the ratio of larval recovery on Bt corn to isoline corn in the field was 11.7-fold greater for the Constant-exposure colony than the Control colony. Removal from selection for six generations did not decrease survival on Bt corn in the greenhouse. The results suggest that rapid response to selection is possible in the absence of mating with unexposed beetles, emphasizing the importance of effective refuges for resistance management.

Artigo completo disponível em http://www.pnas.org/content/105/49/19177.full

Tabashnik, B.; Unnithan, G.; Masson, L.; Crowder, D.; Li, X.; Carrière, Y. 2009. Asymmetrical cross-resistance between Bacillus thuringiensis toxins Cry1Ac and Cry2Ab in pink bollworm. *PNAS USA*, vol. 106, nº 29.

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins kill some key insect pests and can reduce reliance on insecticide sprays. Sustainable use of such crops requires methods for delaying evolution of resistance by pests. To thwart pest resistance, some transgenic crops produce 2 different Bt toxins targeting the same pest. This "pyramid" strategy is expected to work best when selection for resistance to 1 toxin does not cause cross-resistance to the other toxin. The most widely used pyramid is transgenic cotton producing Bt toxins Cry1Ac and Cry2Ab. Cross-resistance between these toxins was presumed unlikely because they bind to different larval midgut target sites. Previous results showed that laboratory selection with Cry1Ac caused little or no cross-resistance to Cry2A toxins in pink bollworm (*Pectinophora gossypiella*), a major cotton pest. We show here, however, that laboratory selection of pink bollworm with Cry2Ab caused up to 420-fold crossresistance to Cry1Ac as well as 240-fold resistance to Cry2Ab. Inheritance of resistance to high concentrations of Cry2Ab was recessive. Larvae from a laboratory strain resistant to Cry1Ac and Cry2Ab in diet bioassays survived on cotton bolls producing only Cry1Ac, but not on cotton bolls producing both toxins. Thus, the asymmetrical cross-resistance seen here does not threaten the efficacy of pyramided Bt cotton against pink bollworm. Nonetheless, the results here and previous evidence indicate that crossresistance occurs between Cry1Ac and Cry2Ab in some key cotton pests. Incorporating the potential effects of such cross-resistance in resistance management plans may help to sustain the efficacy of pyramided Bt crops.

Artigo completo disponível em http://www.pnas.org/content/106/29/11889.full.pdf+html

Oswald, K.; French, B.; Nielson C.; Bagley, M. 2011. Selection for Cry3Bb1 Resistance in a genetically diverse population of nondiapausing western corn rootworm (*Coleoptera: Chrysomelidae*). *Journal of Economic Entomology*, 104: 1038-1044.

Five short-diapause laboratory lines of western corn rootworm, Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), were selected for resistance to MON863, a variety of corn genetically modified with the Bacillus thuringiensis Berliner (Bt) transgene that expresses the Cry3Bb1 delta-endotoxin. Three of the selected lines were developed by incremental increase in the duration of exposure to MON863 over 11 generations (moderate selected lines). Two selected lines were developed from a control group by constant exposure to MON863 for at least 14 d posthatch over seven generations (intense selected lines). At the end of the experiment, survivorship, as measured by adult emergence, was approximately 4 times higher in each of the selected lines reared on MON863 compared with control lines. Estimates of realized heritabilities (h2) were 0.16 and 0.15 for the moderate and intense selected lines, respectively, and are consistent with h2 estimates reported previously from a variety of pest insects. These lines provide data necessary for evaluating the potential for Bt resistance within diabroticite beetles and will be useful for developing improved insect resistance management strategies.

http://www.ncbi.nlm.nih.gov/pubmed/21735927

Frank, D.; Zukoff, A.; Barry, J.; Higdon, M.; Hibbard, B. 2013. Development of Resistance to eCry3.1Ab-Expressing Transgenic Maize in a Laboratory-Selected Population of Western Corn Rootworm (Coleoptera: Chrysomelidae*). Journal of Economic Entomology,* 106(6):2503-2513.

A laboratory colony of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, was selected for resistance to transgenic maize expressing the eCry3.1Ab protein. The selected colony was developed by rearing larvae on nonelite noncommercial Bt maize expressing the eCry3.1Ab protein.

> After four generations, selected and control colonies were screened on eCry3.1Ab-expressing and isoline maize using greenhouse experiments. There was a significant colony × maize pedigree interaction in terms of the number of larvae recovered. There was no significant difference in the number of larvae recovered from eCry3.1Ab-expressing and isoline maize for the selected colony, whereas this difference was significant for the control colony. There was not a significant colony × maize pedigree interaction in terms of root damage, or the number of beetles recovered, but the effect of maize pedigree was significant. After four and eight generations of selection, seedling bioassays were performed. Again, there was a significant colony × maize pedigree interaction in terms of the number of larvae recovered. After 11 generations of selection, larvae from the selected colony had higher LC_{50} values than the control colony when exposed to increasing concentrations of the eCry3.1Ab protein. The resistance ratio of the selected colony was 2.58. These data provide necessary information for understanding the potential for Bt resistance by western corn rootworm and underscores the need for insect resistance management plans for this pest.
>
> http://www.bioone.org/doi/abs/10.1603/EC13148?af=R

2.1.2 Casos de populações de insetos resistentes às toxinas Bt observados no campo

Populações de insetos totalmente insensíveis às toxinas Bt já existem em cinco das maiores espécies consideradas pragas (esse número pelo menos duplica quando se levam em conta as populações pouco sensíveis), considerando as quatro principais proteínas inseticidas (Cry1Ab, Cry1Ac, Cry1F e Cry3Bb1). Enquanto as primeiras populações foram confirmadas nos EUA no início dos anos 2000, insetos resistentes são hoje observados em todos os países grandes produtores de transgênicos, inclusive no Brasil. Essa "quebra de resistência, que tem sido responsabilizada por ondas de suicídio entre cotonicultores da Índia, já levou associações de produtores brasileiros a ingressar na justiça para denunciar propaganda enganosa das empresas de biotecnologias que continuam a vender essas variedades transgênicas como "resistentes a insetos".

Cabe mencionar que os mecanismos biológicos pelos quais os insetos considerados pragas desenvolvem essa resistência ao Bt são diversos, envolvendo um conjunto de moléculas e mutações genéticas. Considerando que novos mecanismos aparecem ao longo do tempo, a quebra de resistência não é algo que poderá ser resolvido com o desenvolvimento de toxinas Bt "diferenciadas" (modificadas para contornar um tipo de mecanismo de resistência).

Shelton, A.; Robertson, J.; Tang, J. 1993. Resistance of diamondback moth (*Lepidoptera: Plutellidae*) to *Bacillus thuringiensis* subspecies in the field. *J. Econ. Entomol.*, 86, 697-705.

Eleven populations of diamondback moth, *Plutella xylostella* (L.), were collected in 1990 from *Brassica* plants in six states of the United States and in Indonesia and tested for their responses to two formulations of *Bacillus thuringiensis* subsp. *Kurstaki* (Javelin we and DipeI2X), permethrin, and methomyI. Populations from Florida that had been treated extensively over several years with these insecticides displayed significantly higher LC50s. In 1992, field tests in geographically separate areas in Florida and laboratory assays of populations from those fields indicated control failures and resistance to products containing *B. thuringiensis* subsp. *kurstaki* and low levels of resistance to a product containing *B. thuringiensis* subsp. *aizawai* (XenTari). These *B. thuringiensis* subspp. differ in the number of toxins produced, but whether resistance to them is a result of cross-resistance or independent selection was not determined. We documented significant differences between the response of resistant and susceptible populations to two products containing *B. thuringiensis* subsp. *kurstaki,* thus suggesting that the products actually differed in the number or amounts of toxins. In laboratory bioassays of three products containing *B. thuringiensis* suhsp. *aizawai* and two products containing *B. thuringiensis* subsp. *kurstaki,* the variation in response (as determined by resistance ratios) varied by 321- to 461-fold for *B. thuringiensis* subsp. *kurstaki* and by 3- to 4.1-fold for *B. thuringiensis* subsp. *aizawai.* These studies indicate increasing resistance problems caused by intensive use of any *B. tlmringiensis* product. We conclude that if *B. thuringiensis* is to remain a durable insecticide in parts of the world where resistance does not already occur, other tactics such as biological control, host-free periods, plant resistance, and cultural controls must he incorporated into the management programs.

Artigo completo disponível em http://web.entomology.cornell.edu/shelton/publications/pdf/Shelton%20et%20al%201993%20Resistance%20of%20diamonback%20moth%20to%20Bt%20subspecies%20in%20the%20field.pdf

Gunning, R.; Dang, H.; Kemp, F.; Nicholson, I.; Moores, G. 2005. New Resistance Mechanism in *Helicoverpa armigera* Threatens Transgenic Crops Expressing *Bacillus thuringiensis* Cry1Ac Toxin. *Applied and Environmental Microbiology*, p. 2558–2563 Vol. 71, No. 5.

In Australia, the cotton bollworm, *Helicoverpa armigera*, has a long history of resistance to conventional insecticides. Transgenic cotton (expressing the *Bacillus thuringiensis* toxin Cry1Ac) has been grown for *H. armigera* control since 1996. It is demonstrated here that a population of Australian *H. armigera* has developed resistance to Cry1Ac toxin (275-fold). Some 70% of resistant *H. armigera* larvae were able to survive on Cry1Ac transgenic cotton (Ingard) The resistance phenotype is inherited as an autosomal semidominant trait. Resistance was associated with elevated esterase levels, which cosegregated with resistance. In vitro studies employing surface plasmon resonance technology and other biochemical techniques demonstrated that resistant strain esterase could bind to Cry1Ac protoxin and activated toxin. In vivo studies showed that Cry1Ac-resistant larvae fed Cy1Ac transgenic cotton or Cry1Ac-treated artificial diet had lower esterase activity than non-Cry1Ac-fed larvae. A resistance mechanism in which esterase sequesters Cry1Ac is proposed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1087549/

Van Rensburg, J. 2007. First report of field resistance by the stem borer, *Busseola fusca* (Fuller) to Bt transgenic maize. *South African Journal of Plant and Soil,* 24(3): 147-151.

Reports of severe damage caused by the African stem borer, *Busseola fusca* (Fuller) to Mon810-transgenic maize (Bt) prompted a study in which the survival of progenies of diapause larvae collected from both a Bt and non-Bt planting were compared when feeding on various Bt and non-Bt hybrids. Field and greenhouse grown plants were artificially infested with neonate larvae. Larval mass was recorded at two-day intervals for three weeks. Data were subjected to simple regression analyses followed by pair wise comparison of the slopes. The two borer populations showed similar larval mass gains on non-Bt hybrids but differed in the response to Bt-hybrids. Appreciable numbers of larvae from the non-Bt derived population survived only to the eighth day. In contrast, substantial numbers of larvae of the Bt-derived population survived over the entire trial period. The mean larval mass of the Bt-derived population at the conclusion of the experiment was less on Bt-hybrids than on their susceptible counterparts. This indicates that the Bt-derived population has attained a level of resistance where some larvae are able to survive in the presence of the Bt-toxin but not without some detrimental effect on larval growth rate. Since producers are inclined to provide refugia under rain fed conditions in the immediate vicinity of irrigated plantings rather than as part of irrigated fields, the known preference of moths for high humidity could have contributed to increased selection pressure towards the development of resistance to the Bt-toxin.

Artigo completo disponível em http://www.tandfonline.com/doi/abs/10.1080/02571862.2007.10634798

Liu, F.; Xu, Z.; Chang, J; Chen, J.; Meng, F.; Zhu, Y.; Shen, J. 2008. Resistance Allele Frequency to Bt Cotton in Field Populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) *China. J. Econ. Entomol.*, 101(3): 933-943.

Resistance evolution in target insects to Bacillus thurningiensis (Bt) cotton, Gossypium hirsutum L., is a main threat to Bt cotton technology. An increasing trend of population density of Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) has been observed since 2001 in Qiuxian County (Hebei, China), where Bt cotton has been planted dominantly since 1998. This region was selected in 2006 and 2007 for estimating frequency of gene alleles conferring resistance to Bt cotton by screening the F1 progeny from single-pair cross between field-collected male and laboratory female of the Bt-resistant strain of H. armigera (F1 screen). F1 offspring from each single-pair line were screened for resistance alleles based on larval growth, development, and survival on Bt cotton leaves for 5 d. Two-year results indicated that approximately equal to 20% of field-collected males carried resistance alleles. The conservative estimate of the resistance allele frequency was 0.094 (95% CI, 0.044-0.145) for 2006 and 0.107 (95% CI, 0.055-0.159) for 2007. This is the first report of resistance allele frequency increase to such a high level in the field in China. Long-term adoption of Bt sprays, dominant planting of single-toxin-producing Bt cotton, and lack of conventional cotton refuge system might accelerate the resistance evolution in the region.

http://www.ncbi.nlm.nih.gov/pubmed/18613597

REV – Tabashnik, B.; van Rensburg, J.; Carrière, Y. 2009. Field-Evolved Insect Resistance to Bt Crops: Definition, Theory, and Data. *J. Econ. Entomol.,* 102(6): 2011-2025.

Transgenic crops producing *Bacillus thuringiensis* (*Bt*) toxins for insect pest control have been successful, but their efÞcacy is reduced when pests evolve resistance. Here we review the deÞnition of Þeld-evolved resistance, the relationship between resistance and Þeld control problems, the theory underlying strategies for delaying resistance, and resistance monitoring methods. We also analyze resistance monitoring data from five continents reported in 41 studies that evaluate responses of field populations of 11 lepidopteran pests to four *Bt* toxins produced by *Bt* corn and cotton. After more than a decade since initial commercialization of *Bt* crops, most target pest populations remain susceptible, whereas field-evolved resistance has been documented in some populations of three noctuid moth species: *Spodoptera frugiperda* (J. E. Smith) to Cry1F in *Bt* corn in Puerto Rico, *Busseola fusca* (Fuller) to Cry1Ab in *Bt* corn in South Africa, and *Helicoverpa zea* (Boddie) to Cry1Ac and Cry2Ab in *Bt* cotton in the southeastern United States. Field outcomes are consistent with predictions from theory, suggesting that factors delaying resistance include recessive inheritance of resistance, abundant refuges of non-*Bt* host plants, and two-toxin *Bt* crops deployed separately from one-toxin *Bt* crops. The insights gained from systematic analyses of resistance monitoring data may help to enhance the durability of transgenic insecticidal crops. We recommend continued use of the longstanding definition of resistance cited here and encourage discussions about which regulatory actions, if any, should be triggered by specific data on the magnitude, distribution, and impact of field-evolved resistance.

http://www.ncbi.nlm.nih.gov/pubmed/20069826

COM – Bagla, P. 2010. Hardy Cotton-Munching Pests Are Latest Blow to GM Crops. *Science*, vol 327.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/20299559

Caccia, S.; Hernández-Rodríguez, C.; Mahon, R.; Downes, S.; James, W.; et al. 2010. Binding site alteration is responsible for field-isolated resistance to *Bacillus thuringiensis* Cry2A insecticidal proteins in two Helicoverpa species. *PLOS ONE,* 5(4): e9975.

Background: Evolution of resistance by target pests is the main threat to the long-term efficacy of crops expressing *Bacillus thuringiensis* (*Bt*) insecticidal proteins. Cry2 proteins play a pivotal role in current Bt spray formulations and transgenic crops and they complement Cry1A proteins because of their different mode of action. Their presence is critical in the control of those lepidopteran species, such as *Helicoverpa* spp., which are not highly susceptible to Cry1A proteins. In Australia, a transgenic variety of cotton expressing Cry1Ac and Cry2Ab (Bollgard II) comprises at least 80% of the total cotton area. Prior to the widespread adoption of Bollgard II, the frequency of alleles conferring resistance to Cry2Ab in field populations of *Helicoverpa armigera* and *Helicoverpa punctigera* was significantly higher than anticipated. Colonies established from survivors of F_2 screens against Cry2Ab are highly resistant to this toxin, but susceptible to Cry1Ac.
Methodology/Principal Findings: Bioassays performed with surface-treated artificial diet on neonates of *H. armigera* and *H. punctigera* showed that Cry2Ab resistant insects were cross-resistant to Cry2Ae while susceptible to Cry1Ab. Binding analyses with ^{125}I-labeled Cry2Ab were performed with brush border membrane vesicles from midguts of Cry2Ab susceptible and resistant insects. The results of the binding analyses correlated with bioassay data and demonstrated that resistant insects exhibited greatly reduced binding of Cry2Ab toxin to midgut receptors, whereas no change in ^{125}I-labeled-Cry1Ac binding was detected. As previously demonstrated for *H. armigera*, Cry2Ab binding sites in *H. punctigera* were shown to be shared by Cry2Ae, which explains why an alteration of the shared binding site would lead to cross-resistance between the two Cry2A toxins.
Conclusion/Significance: This is the first time that a mechanism of resistance to the Cry2 class of insecticidal proteins has been reported. Because we found the same mechanism of resistance in multiple strains representing several field populations, we conclude that target site alteration is the most likely means that field populations evolve resistance to Cry2 proteins in *Helicoverpa* spp. Our work also confirms the presence in the insect midgut of specific binding sites for this class of proteins. Characterizing the Cry2 receptors and their mutations that enable resistance could lead to the development of molecular tools to monitor resistance in the field.

Artigo completo disponível em http://www.plosone.org/article/fetchObject.action?uri=info%3Adoi%2F10.1371%2Fjournal.pone.0009975&representation=PDF

Storer, N.; Babcock, J.; Schlenz, M.; Meade, T.; Thompson, G.; Bing, J.; Huckaba, R. 2010. Discovery and characterization of field resistance to Bt maize: *Spodoptera frugiperda* (*Lepidoptera: Noctuidae*) in Puerto Rico. *J Econ Entomol*., 103(4): 1031-8.

Transgenic maize, Zea mays L., event TC1507 produces the Cry1F protein to provide protection from feeding by several important lepidopteran pests, including Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae). Reports of reduced field performance against this species in Puerto Rico were investigated, and laboratory bioassays showed that S. frugiperda collected from the affected area exhibited lower sensitivity to the Cry1F protein compared with typical colonies from other regions. The resistance was shown to be autosomally inherited and highly recessive. The Puerto Rico colony was shown to be moderately less sensitive than susceptible laboratory strains to Cry1Ab and Cry1Ac, but the differences in sensitivity were dramatically smaller than for Cry1F. Potential contributory factors to the emergence of resistance to Cry1F in Puerto Rico populations of S. frugiperda include the tropical island geography, unusually large population sizes in 2006, and drought conditions reducing the availability of alternative hosts. In response to this resistance incident, the technology providers have stopped commercial sales of TC1507 maize in Puerto Rico pending potential reversion to susceptibility.

http://www.ncbi.nlm.nih.gov/pubmed/20857709

Ranjith, M.; Prabhuraj, A.; Srinivasa, Y. 2010. Survival and reproduction of natural populations of Helicoverpa armigera on Bt-cotton hybrids in Raichur, India. *Current Science*, vol. 99 (11).

Transgenic *Bt*-cotton is commercially cultivated on the rationale that it produces toxins that defend the plants primarily from caterpillars damaging cotton bolls. From the context of crop protection, it is important that these bollworms remain susceptible to the toxins, so that their populations are under check. However, if certain individuals are able to survive and breed on the transgenics, they can build populations resistant to the toxins. In one such instance we discovered individuals of *Helicoverpa armigera*, the most prominent among bollworms in India, surviving on commercial *Bt*-cotton hybrids containing single (*Cry*1Ac) and double (*Cry*1Ac and *Cry*2Ab) genes in experimental plots of the University of Agricultural Sciences, Raichur campus, India. Analyses of various biological parameters measured through laboratory breeding on the respective hybrids revealed that these surviving individuals could not only complete their life cycle but also reproduce. A proportion of individuals of the succeeding generation were also able to complete their life cycle on the transgenic commercial hybrids. Interestingly, many of the biological parameters of the bollworm across *Bt* and non-*Bt* hybrids were mostly comparable. These results not only validate the occurrence of natural populations of *H. armigera* on *Bt* cotton hybrids, but also provide evidence for its survival and successful reproduction in India.

http://www.biosafety-info.net/article.php?aid=753

Gassmann, A.; Petzold-Maxwell, J.; Keweshan, R.; Dunbar, M. 2011. Field evolved resistance to Bt maize by Western Corn Rootworm. *PLOS ONE,* 6(7): 222629.

The widespread planting of crops genetically engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) places intense selective pressure on pest populations to evolve resistance. Western corn rootworm is a key pest of maize, and in continuous maize fields it is often managed through planting of Bt maize. During 2009 and 2010, fields were identified in Iowa in which western corn rootworm imposed severe injury to maize producing Bt toxin Cry3Bb1. Subsequent bioassays revealed Cry3Bb1 resistance in these populations. Here, we report that, during 2011, injury to Bt maize in the field expanded to include mCry3A maize in addition to Cry3Bb1 maize and that laboratory analysis of western corn rootworm from these fields found resistance to Cry3Bb1 and mCry3A and cross-resistance between these toxins. Resistance to Bt maize has persisted in Iowa, with both the number of Bt fields identified with severe root injury and the ability western corn rootworm populations to survive on Cry3Bb1 maize increasing between 2009 and 2011. Additionally, Bt maize targeting western corn rootworm does not produce a high dose of Bt toxin, and the magnitude of resistance associated with feeding injury was less than that seen in a high-dose Bt crop. These first cases of resistance by western corn rootworm highlight the vulnerability of Bt maize to further evolution of resistance from this pest and, more broadly, point to the potential of insects to develop resistance rapidly when Bt crops do not achieve a high dose of Bt toxin.

Artigo completo disponível em http://www.pnas.org/content/111/14/5141.full

Dhurua, S.; Gujar, G. 2011. Field-evolved resistance to Bt toxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (*Lepidoptera: Gelechiidae*) from India. *Pest Management Science,*vol. 67 (8): 898-903.

Background: The pink bollworm is one of the most destructive pests of cotton. Transgenic cotton producing Bt toxin Cry1Ac or a combination of Cry1Ac and Cry2Ab2 has been used effectively against this pest. However, some other insects have evolved resistance to Bt toxins in the field. During the 2007-2008 and 2008-2009 seasons, pink bollworm populations in India were surveyed to evaluate their responses to Cry1Ac and seed powder containing Cry1Ac and Cry2Ab2.
Results: The results provide evidence that resistance to Cry1Ac had evolved by 2008 in a population sampled from non-Bt cotton in the Amreli district of Gujarat in western India. The median lethal concentration of Cry1Ac for five-day-old larvae (LC50) was significantly higher for insects derived in 2008 from Amreli than for any of the other field populations tested from four locations in India. For Cry1Ac, the mean LC50 for the strain derived from Amreli in 2008 was 44 times higher than for the most susceptible population. However, for seed powder of Bollgard II containing primarily Cry2Ab2, the 2008 Amreli population was only slightly less susceptible than the most susceptible population.
Conclusions: The data reported here constitute the first evidence of field-evolved resistance of pink bollworm to Cry1Ac. This initial evidence spurred more extensive evaluations during the 2009-2010 growing season, which confirmed field-evolved resistance to Cry1Ac in Amreli. The lack of cross-resistance to Cry2Ab2 suggests that plants producing this toxin are likely to be more effective against resistant populations than plants producing only Cry1Ac.

http://www.ncbi.nlm.nih.gov/pubmed/21438121

Tabashnik, B.; Wu, K.; Wu, Y. 2012. Early detection of field-evolved resistance to Bt cotton in China: cotton bollworm and pink bollworm. J Invertebr Pathol., 110(3):301-6. doi: 10.1016/j.jip.2012.04.008. Epub 2012 Apr 16.

Transgenic crops producing Bacillus thuringiensis (Bt) toxins kill some major insect pests, but pests can evolve resistance and thereby reduce the effectiveness of such Bt crops. The main approach for slowing pest adaptation to Bt crops uses non-Bt host plants as “refuges” to increase survival of susceptible pests. To delay evolution of pest resistance to cotton producing Bt toxin Cry1Ac, several countries have required refuges of non-Bt cotton, while farmers in China have relied on “natural” refuges of non-Bt host plants other than cotton. This strategy is designed for cotton bollworm (Helicoverpa armigera), which attacks many crops and is the primary target of Bt cotton in China, but it does not apply to pink bollworm (Pectinophora gossypiella), which feeds almost entirely on cotton in China. Here we review evidence of field-evolved resistance to Cry1Ac by cotton bollworm in northern China and by pink bollworm in the Yangtze River Valley of China. For both pests, results of laboratory diet bioassays reveal significantly decreased susceptibility of field populations to Cry1Ac, yet field control failures of Bt cotton have not been reported. The early detection of resistance summarized here may spur countermeasures such as planting Bt cotton that produces two or more distinct toxins, increased planting of non-Bt cotton, and integration of other management tactics together with Bt cotton.

http://www.ncbi.nlm.nih.gov/pubmed/22537835

REV – Tabashnik, B.; Brévault, T.; Carrière, Y. 2013. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology*, 31, 510–521 doi:10.1038/nbt.2597.

Evolution of resistance in pests can reduce the effectiveness of insecticidal proteins from *Bacillus thuringiensis* (Bt) produced by transgenic crops. We analyzed results of 77 studies from five continents reporting field monitoring data for resistance to Bt crops, empirical evaluation of factors affecting resistance or both. Although most pest populations remained susceptible, reduced efficacy of Bt crops caused by field-evolved resistance has been reported now for some populations of 5 of 13 major pest species examined, compared with resistant populations of only one pest species in 2005. Field outcomes support theoretical predictions that factors delaying resistance include recessive inheritance of resistance, low initial frequency of resistance alleles, abundant refuges of non-Bt host plants and two-toxin Bt crops deployed separately from one-toxin Bt crops. The results imply that proactive evaluation of the inheritance and initial frequency of resistance are useful for predicting the risk of resistance and improving strategies to sustain the effectiveness of Bt crops.

http://www.ncbi.nlm.nih.gov/pubmed/23752438

Farias, J.; Andow, D.; Horikoshi, R.; Sorgatto, R.; Fresia, P; Santos, A.; Omoto, C. 2014. Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda (Lepidoptera: Noctuidae*) in Brazil. *Crop Protection*, volume 64, Pages 150–158.

The Cry1F protein from *Bacillus thuringiensis* Berliner expressed in event TC1507 maize (*Zea mays* L.) was one of the most effective ways to control *Spodoptera frugiperda* (J. E. Smith) in Brazil. After reports of reduced effectiveness of this Bt maize event in some areas of Brazil, research was undertaken to investigate if damage to Cry1F maize was caused by resistant *S. frugiperda*. Additional investigations were conducted to evaluate the genetic basis of the resistance and to test if Cry1F resistant *S. frugiperda* selected from populations of different regions of Brazil share the same resistance locus by using complementation tests. Neonate larvae of *S. frugiperda* collected from TC1507 maize fields with damage in Western Bahia region in 2011 were able to survive on Cry1F maize plants under laboratory conditions and subsequently produced normal adults. Survival of Cry1F-susceptible *S. frugiperda* on non-Bt maize was significantly higher in leaf than plant bioassays. Resistance ratio in diet overlay bioassays was >5000-fold. A discriminating concentration of 2000 ng cm^{-2} of Cry1F protein was defined for monitoring the frequency of resistance of *S. frugiperda* to Cry1F. Cry1F resistant *S. frugiperda* showed a recessive autosomal inheritance for alleles involved in resistance to Cry1F protein. In complementation tests, the resistant population from Western Bahia was crossed with the other seven resistant populations collected from different States of Brazil. F$_1$ larvae from each cross had the same survival at discriminating concentration of 2000 ng cm^{-2} of Cry1F protein, indicating that the resistance alleles in each population were likely at the same locus. Therefore, implementation of resistance management strategies is urgent to prolong the lifetime of Cry1F for controlling *S. frugiperda* in Brazil.

http://www.sciencedirect.com/science/article/pii/S026121941400204X

Gassmann, A.; Petzold-Maxwell, J.; Clifton, E.; Dunbar, M.; Hoffmann, A.; Ingber, D.; Keweshan, R. 2014. Field-evolved resistance by western corn rootworm to multiple *Bacillus thuringiensis* toxins in transgenic maize. *PNAS* USA, vol. 111, no. 14, 5141–5146.

The widespread planting of crops genetically engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (Bt) places intense selective pressure on pest populations to evolve resistance. Western corn rootworm is a key pest of maize, and in continuous maize fields it is often managed through planting of Bt maize. During 2009 and 2010, fields were identified in Iowa in which western corn rootworm imposed severe injury to maize producing Bt toxin Cry3Bb1. Subsequent bioassays revealed Cry3Bb1 resistance in these populations. Here, we report that, during 2011, injury to Bt maize in the field expanded to include mCry3A maize in addition to Cry3Bb1 maize and that laboratory analysis of western corn rootworm from these fields found resistance to Cry3Bb1 and mCry3A and cross-resistance between these toxins. Resistance to Bt maize has persisted in Iowa, with both the number of Bt fields identified with severe root injury and the ability western corn rootworm populations to survive on Cry3Bb1 maize increasing between 2009 and 2011. Additionally, Bt maize targeting western corn rootworm does not produce a high dose of Bt toxin, and the magnitude of resistance associated with feeding injury was less than that seen in a high-dose Bt crop. These first cases of resistance by western corn rootworm highlight the vulnerability of Bt maize to further evolution of resistance from this pest and, more broadly, point to the potential of insects to develop resistance rapidly when Bt crops do not achieve a high dose of Bt toxin.

Artigo completo disponível em http://www.pnas.org/content/111/14/5141.full.pdf+html

Fabrick, J.; Ponnuraj, J.; Singh, A.; Tanwar, R.; Unnithan, G.; Yelich, A.; Li, X.; Carrière, Y.; Tabashnik, B. 2014. Alternative splicing and highly variable cadherin transcripts associated with field-evolved resistance of pink bollworm to Bt cotton in India. *PLOS ONE,* 19;9(5):e97900. doi: 10.1371/journal.pone.0097900. eCollection 2014.

Evolution of resistance by insect pests can reduce the benefits of insecticidal proteins from Bacillus thuringiensis (Bt) that are used extensively in sprays and transgenic crops. Despite considerable knowledge of the genes conferring insect resistance to Bt toxins in laboratory-selected strains and in field populations exposed to Bt sprays, understanding of the genetic basis of field-evolved resistance to Bt crops remains limited. In particular, previous work has not identified the genes conferring resistance in any cases where field-evolved resistance has reduced the efficacy of a Bt crop. Here we report that mutations in a gene encoding a cadherin protein that binds Bt toxin Cry1Ac are associated with field-evolved resistance of pink bollworm (Pectinophora gossypiella) in India to Cry1Ac produced by transgenic cotton. We conducted laboratory bioassays that confirmed previously reported resistance to Cry1Ac in pink bollworm from the state of Gujarat, where Bt cotton producing Cry1Ac has been grown extensively. Analysis of DNA from 436 pink bollworm from seven populations in India detected none of the four cadherin resistance alleles previously reported to be linked with resistance to Cry1Ac in laboratory-selected strains of pink bollworm from Arizona. However, DNA sequencing of pink bollworm derived from resistant and susceptible field populations in India revealed eight novel, severely disrupted cadherin alleles associated with resistance to Cry1Ac. For these eight alleles, analysis of complementary DNA (cDNA) revealed a total of 19 transcript isoforms, each containing a premature stop codon, a deletion of at least 99 base pairs, or both. Seven of the eight disrupted alleles each produced two or more different transcript isoforms, which implicates alternative splicing of messenger RNA (mRNA). This represents the first example of alternative splicing associated with field-evolved resistance that reduced the efficacy of a Bt crop.

Artigo completo disponível em http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0097900

Além da crescente presença de insetos insensíveis às toxinas Bt, alguns pesquisadores observaram que esses indivíduos podem possuir maior *fitness* do que seus congêneres de populações sensíveis. Assim, alguns insetos que adquirem resistência acabam se reproduzindo de modo mais eficiente, ampliando rapidamente suas populações e gerando danos agronômicos e socioeconômicos potencialmente mais relevantes do que aqueles observados em sua ausência.

Oswald, K.; French, B.; Nielson, C.; Bagley, M. 2012. Assessment of fitness costs in Cry3Bb1-resistant and susceptible western corn rootworm (*Coleoptera: Chrysomelidae*) laboratory colonies. *Journal of Applied Entomology,* Doi: 10.1111/j.1439-0418.2012.01704.x

Maize production in the United States is dominated by plants genetically modified with transgenes from *Bacillus thuringiensis* (Bt). Cry3Bb delta endotoxins expressed by Bt maize specifically target corn rootworms (genus *Diabrotica*) and have proven highly efficacious. However, development of resistance to Bt maize, especially among western corn rootworm (*Diabrotica virgifera virgifera*) populations, poses a significant threat to the future viability of this pest control biotechnology. The structured refuge insect resistance management (IRM) strategy implemented in the United States for Bt maize adopts a conservative approach to managing resistance by assuming no fitness costs of Bt resistance, even though these trade-offs strongly influence the dynamics of Bt resistance within numerous agricultural pest species. To investigate the effects of Bt resistance on fitness components of western corn rootworm, we compared survivorship, fecundity and viability of five Bt-resistant laboratory lines reared on MON863 (YieldGard Rootworm), a Bt maize product that expresses Cry3Bb1 delta endotoxin, and on its non-transgenic isoline. Analysis of performance on the isoline maize demonstrated no fitness costs associated with Bt resistance. In fact, resistant lines emerged approximately 2–3 days earlier than control lines when reared on both MON863 and the isoline, indicating that selection for Bt resistance resulted in a general increase in the rate of larval development. In addition, resistant lines reared on Bt maize displayed higher fecundity than those reared on the isoline, which may have significant management implications. These data will be valuable for formulating improved IRM strategies for a principal agricultural pest of maize.

http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=238506&fed_org_id=770&SIType=PR&TIMSType=&showCriteria=0&address=nerl/pubs.html&view=citation&personID=2915&role=Author&sortBy=pubDateYear&count=100&dateBeginPublishedPresented=2.1.3 Baixa eficácia das estratégias de manutenção da vida útil da tecnologia Bt

2.1.3 Baixa eficácia das estratégias de manutenção da vida útil da tecnologia Bt

Logo após o registro das primeiras populações de insetos geneticamente resistentes a toxinas Bt, foram desenvolvidas estratégias de manejo objetivando estender o tempo de eficácia da tecnologia Bt. Com preocupação diretamente relacionada à manutenção das taxas de lucro, com vistas a estender a vida útil da tecnologia, foram propostos mecanismos que transferem para o agricultor a responsabilidade por condição natural, decorrente da presença opressiva das proteínas Cry. Nesse sentido, duas estratégias vêm sendo recomendadas aos produtores de plantas transgênicas Bt pelo setor das biotecnologias.

A primeira estratégia envolve as empresas que desenvolvem biotecnologias e os produtores. As empresas passam a comercializar plantas Bt que sintetizam – supostamente[30] – altas doses de toxinas (no

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[30] Variações na síntese de proteínas Cry por plantas Bt são exemplificadas no item 2.2.2 da Parte 1.

objetivo de maximizar as possibilidades de morte dos insetos[31]), cabendo aos produtores implantar as chamadas zonas de refúgio. Próximas a suas lavouras, esses refúgios correspondem a parcelas de áreas de cultivo semeadas com variedades não geneticamente modificadas. Seria uma espécie de semeadura "para os insetos", de modo a manter predominância numérica de populações sensíveis. Ora, a resistência dos agricultores seria de esperar, com todos aguardando que os vizinhos realizassem os cultivos antieconômicos das áreas de refúgio, que serviriam basicamente para preservar os lucros das empresas. Entretanto, maiores cargas de proteínas inseticidas não impediram ou mesmo retardaram a emergência de populações tolerantes. Além disto, a estratégia de refúgios se mostrou frágil, já que, em muitos casos – em função das espécies e das predisposições de determinadas populações em ser selecionadas –, mais da metade da lavoura necessitaria ser plantada com variedades convencionais para se obter a manutenção da eficácia da tecnologia durante alguns anos.

MOD-REV – Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annu Rev Entomol.*, 43: 701-26.

This review examines potential impacts of transgenic cultivars on insect population dynamics and evolution. Experience with classically bred, insecticidal cultivars has demonstrated that a solid understanding of both the target insect's ecology and the cultivar's performance under varied field conditions will be essential for predicting area-wide effects of transgenic cultivars on pest and natural enemy dynamics. This experience has also demonstrated the evolutionary capacity of pests for adaptive response to insecticidal traits in crops. Biochemical and genetic studies of insect adaptation to the *Bacillus thuringiensis* (Bt) toxins expressed by currently marketed transgenic cultivars indicate a high risk for rapid adaptation if these cultivars are misused. Theoretical and practical issues involved in implementing strategies to delay pest adaptation to insecticidal cultivars are reviewed. Emphasis is placed on examining the "high dose"/refuge strategy that has become the goal of industry and regulatory authorities.

http://www.ncbi.nlm.nih.gov/pubmed/15012402

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[31] Um dos pré-requisitos para eficácia da estratégia de alta dose/refúgio é que as mutações genéticas responsáveis pela insensibilidade à toxina sejam transmitidas de modo recessivo (a insensibilidade será exprimida na próxima geração apenas se o inseto acasalar com indivíduo também dotado da mutação). Ora, determinadas pragas agronomicamente importantes possuem transmissão não recessiva (ou dominante) desse caractere, invalidando essa estratégia de manejo.

Huang, F.; Buschman, L.; Higgins, R.; McGaughey, W. 1999. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science,* 284: 965-966.

Resistance in the European corn borer, *Ostrinia nubilalis* (Hu¬bner), to a commercial formulation of *Bacillus thuringiensis* (*Bt*) Berliner toxin, Dipel ES, appears to be inherited as an incompletely dominant autosomal gene. This contrasts with the inheritance of resistance to *Bt* in other insects, where it has usually been characterized as a recessive trait. The proposed high-dose/refuge strategy for resistance management in *Bt* maize depends on resistance being recessive or partially recessive. If field resistance turns out to be similar to this laboratory resistance, the usefulness of the high-dose/refuge strategy for resistance management in *Bt* maize may be diminished.

http://www.ncbi.nlm.nih.gov/pubmed/10320377

Shelton, A.; Tang, J.; Roush, R.; Metz, T.; Earle, E. 2000. Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnology*, vol 18.

Several important crops have been engineered to express toxins of *Bacillus thuringiensis* (*Bt*) for insect control. In 1999, US farmers planted nearly 8 million hectares (nearly 20 million acres) of transgenic *Bt* crops approved by the EPA. *Bt*-transgenic plants can greatly reduce the use of broader spectrum insecticides, but insect resistance may hinder this technology. Present resistance management strategies rely on a "refuge" composed of non-*Bt* plants to conserve susceptible alleles. We have used *Bt*-transgenic broccoli plants and the diamondback moth as a model system to examine resistance management strategies. The higher number of larvae on refuge plants in our field tests indicate that a "separate refuge" will be more effective at conserving susceptible larvae than a "mixed refuge" and would thereby reduce the number of homozygous resistant (RR) offspring. Our field tests also examined the strategy of spraying the refuge to prevent economic loss to the crop while maintaining susceptible alleles in the population. Results indicate that great care must be taken to ensure that refuges, particularly those sprayed with efficacious insecticides, produce adequate numbers of susceptible alleles. Each insect/*Bt* crop system may have unique management requirements because of the biology of the insect, but our studies validate the need for a refuge. As we learn more about how to refine our present resistance management strategies, it is important to also develop the next generation of technology and implementation strategies.

http://www.ncbi.nlm.nih.gov/pubmed/10700153

REV – Tabashnik, B.; Carrière, Y.; Dennehy, T.; Morin, S.; Sisterson, M.; Roush, R. Shelton, A.; Zhao, J-Z. 2003. Insect Resistance to Transgenic Bt Crops: Lessons from the Laboratory and Field. *J. Econ. Entomol.*, 96(4): 1031-1038.

Transgenic crops that produce insecticidal toxins from the bacterium Bacillus thuringiensis (Bt) grew on >62 million ha worldwide from 1996 to 2002. Despite expectations that pests would rapidly evolve resistance to such Bt crops, increases in the frequency of resistance caused by exposure to Bt crops in the field have not yet been documented. In laboratory and greenhouse tests, however, at least seven resistant laboratory strains of three pests (Plutella xylostella [L.], Pectinophora gossypiella [Saunders], and Helicoverpa armigera [Hübner]) have completed development on Bt crops. In contrast, several other laboratory strains with 70- to 10,100-fold resistance to Bt toxins in diet did not survive on Bt crops. Monitoring of field populations in regions with high adoption of Bt crops has not yet detected increases in resistance frequency. Resistance monitoring examples include Ostrinia nubilalis (Hübner) in the United States (6 yr), P. gossypiella in Arizona (5 yr), H. armigera in northern China (3 yr), and Helicoverpa zea (Boddie) in North Carolina (2 yr). Key factors delaying resistance to Bt crops are probably refuges of non-Bt host plants that enable survival of susceptible pests, low initial resistance allele frequencies, recessive inheritance of resistance to Bt crops, costs associated with resistance that reduce fitness of resistant individuals relative to susceptible individuals on non-Bt hosts ("fitness costs"), and disadvantages suffered by resistant strains on Bt hosts relative to their performance on non-Bt hosts ("incomplete resistance"). The relative importance of these factors varies among pest-Bt crop systems, and violations of key assumptions of the refuge strategy (low resistance allele frequency and recessive inheritance) may occur in some cases. The success of Bt crops exceeds expectations of many, but does not preclude resistance problems in the future.

http://www.ncbi.nlm.nih.gov/pubmed/14503572

Chilcutt, C.; Tabashnik, B. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. *Proceedings of the National Academy of Sciences,* 101:7526-7529.

Transgenic crops producing insecticidal toxins from *Bacillus thuringiensis* (Bt) are widely used to control pests, but their benefits will be lost if pests evolve resistance. The mandated high-dose/refuge strategy for delaying pest resistance requires planting refuges of toxin-free crops near Bt crops to promote survival of susceptible pests. We report that pollen-mediated gene flow up to 31 m from Bt maize caused low to moderate Bt toxin levels in kernels of non-Bt maize refuge plants. Immunoassays of non-Bt maize sampled from the field showed that the mean concentration of Bt toxin Cry1Ab in kernels and the percentage of kernels with Cry1Ab decreased with distance from Bt maize. The highest Bt toxin concentration in pooled kernels of non-Bt maize plants was 45% of the mean concentration in kernels from adjacent Bt maize plants. Most previous work on gene flow from transgenic crops has emphasized potential effects of transgene movement on wild relatives of crops, landraces, and organic plantings, whereas implications for pest resistance have been largely ignored. Variable Bt toxin production in seeds of refuge plants undermines the high dose/refuge strategy and could accelerate pest resistance to Bt crops. Thus, guidelines should be revised to reduce gene flow between Bt crops and refuge plants.

Artigo completo disponível em http://www.pnas.org/content/101/20/7526.full?sid=5acea452-f319-4f56-94d5-9f4911ea842d

MOD – Kranthi, K.; Kranthi, N. 2004. Modelling adaptability of cotton bollworm, *Helicoverpa armigera* (Hübner) to Bt-cotton in India. *Current Science,* vol. 87, nº 8.

A stochastic model '*Bt*-Adapt' was developed to simulate the rate of resistance development of *Helicoverpa armigera* to Cry1Ac under Indian farming conditions. The model integrates genetic and ecological parameters of *H. armigera* in relation to its response to the Cry1Ac expressing *Bacillus thuringiensis* (*Bt*)-cotton. Simulation analysis showed that relative survival rate of the Cry1Ac-resistant homozygous (RR), heterozygous (RS) and homozygous susceptible (SS) *H. armigera* genotypes on *Bt*-cotton, was the most important factor influencing resistance development. In the order of significance, the other factors that had the greatest impact on resistance development were the relative proportion of area under *Bt*-cotton, dominance of the resistant allele and initial frequency of resistant alleles in field populations. The extent of population reduction in *Bt*-cotton and non-*Bt* crops due to pest control, was found to have a significant impact on the rate of resistance development. Simulation studies showed that cultivation of *Bt*-cotton in 10, 20, 30 and 40% of the total area under cotton, is likely to result in resistant allele frequency reaching 0.5, which would be adequate to cause crop failure, after 54, 25, 16 and 11 years respectively, if no pest control measures were adopted in both *Bt*-cotton and non-*Bt* crops. With a pest control efficacy of 0.9 in *Bt*-cotton and 0.5 in non- *Bt* crops, it would take 70 and 45 years for resistant allele frequency to reach 0.5 with the *Bt*-cotton area at 30 and 40% respectively. Based on the simulation analysis, resistance management strategies are proposed with emphasis on reducing populations of *H.armigera* that survive *Bt*-cotton and enhancement of area of alternate host crops that are as attractive as cotton to *H. armigera*, to be used as trap crop or intercrop refuges.

http://4ccr.pgr.mpf.mp.br/institucional/grupos-de-trabalho/gt-transgenicos/documentos-diversos/bibliografia/pgm-resultados-contestados/Kranthi%20et%20al,%202004,%20Cur%20Sci.pdf

REV – Tabashnik, B.; Gassmann, A.; Crowder, D.; Carrière, Y. 2008. Insect resistance to Bt crops: Evidence versus Theory. *Nature Biotechnology,* 26: 199-202.

Evolution of insect resistance threatens the continued success of transgenic crops producing *Bacillus thuringiensis* (*Bt*) toxins that kill pests. The approach used most widely to delay insect resistance to *Bt* crops is the refuge strategy, which requires refuges of host plants without *Bt* toxins near *Bt* crops to promote survival of susceptible pests. However, large-scale tests of the refuge strategy have been problematic. Analysis of more than a decade of global monitoring data reveals that the frequency of resistance alleles has increased substantially in some field populations of *Helicoverpa zea*, but not in five other major pests in Australia, China, Spain and the United States. The resistance of *H. zea* to *Bt* toxin Cry1Ac in transgenic cotton has not caused widespread crop failures, in part because other tactics augment control of this pest. The field outcomes documented with monitoring data are consistent with the theory underlying the refuge strategy, suggesting that refuges have helped to delay resistance.

http://www.ncbi.nlm.nih.gov/pubmed/18259177

COM – Tabashnik, B. 2008. Delaying insect resistance to transgenic crops. *PNAS USA*, 105 (49) 19029-19030, vol. 105, nº 49 19029–19030 – 1.

Sem resumo.

Artigo completo disonível em http://www.pnas.org/content/105/49/19029.full.pdf+html

Kruger, M.; van Rensburg, J.; Van den Berg, J. 2009. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. *Crop Protection,* 28 (8): 684-9.

Bt maize has been grown at the Vaalharts irrigation scheme in South Africa since its first release during 1998. Interest in Bt maize refuge compliance, pest incidence and production practices at Vaalharts were recently stimulated by the first report of field resistance of Busseola fusca (Lepidoptera: Noctuidae) to Bt maize. Objectives of this study were to evaluate farmer's perceptions of the regulatory aspects guiding the planting of Bt maize and refugia and how the field situation developed between 1998 and 2008. A survey, using a self-administered questionnaire, was conducted amongst 80 farmers at the irrigation scheme. The questionnaire addressed signing of contracts upon purchasing genetically modified (GM) seed, refuge compliance, refuge design and general farming practices. Farmers were also questioned on the perceived benefits and disadvantages of Bt maize and their perceptions of the pest status of B. fusca. The two greatest advantages associated with Bt maize were indicated to be convenient management (88%) and increased productivity (61.3%) while 42.5% indicated that they perceived Bt-technology to be environmental friendly. Initial levels of refuge compliance were low, and even though farmers were obligated to plant a refuge area for each Bt maize field, only 7.7% of farmers planted refuges during 1998. This number increased to 100% during 2008. Eight percent of farmers, however, indicated that they did not plant a refuge field for each Bt maize field, which was justified on the basis of small farm sizes (25 ha). Nearly all farmers (99.8%) allow no spatial separation between the Bt maize field and adjacent refuge area. Farmers preferred to plant the refuge option where 5% of the field area is planted to conventional maize, which is not sprayed with insecticide instead of the 20% refuge area on which insecticide application against the target pest is allowed. In South Africa stewardship programs instituted during the 2008/2009 growing season, involve grower education programs as well as the compulsory signing of contracts between companies and farmers that contractually bind them to comply with refuge requirements accompanied by on-farm inspections. It appears that stem borer resistance to Bt maize in the Vaalharts area resulted from a combination of a late general planting date with consequent increased levels of infestation and variance in time of planting providing a continuous supply of moths.

http://www.sciencedirect.com/science/article/pii/S0261219409000921

Carrière, Y.; Crowder, D.; Tabashnik, B. 2010. Evolutionary ecology of insect adaptation to Bt crops. *Evolutionary Applications* (Special Issue: Evolution in Agro-Ecosystems) vol. 3, Issue 5-6: 561-73.

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins are used worldwide to control major pests of corn and cotton. Development of strategies to delay the evolution of pest resistance to Bt crops requires an understanding of factors affecting responses to natural selection, which include variation in survival on Bt crops, heritability of resistance, and fitness advantages associated with resistance mutations. The two main strategies adopted for delaying resistance are the refuge and pyramid strategies. Both can reduce heritability of resistance, but pyramids can also delay resistance by reducing genetic variation for resistance. Seasonal declines in the concentration of Bt toxins in transgenic cultivars, however, can increase the heritability of resistance. The fitness advantages associated with resistance mutations can be reduced by agronomic practices, including increasing refuge size, manipulating refuges to increase fitness costs, and manipulating Bt cultivars to reduce fitness of resistant individuals. Manipulating costs and fitness of resistant individuals on transgenic insecticidal crops may be especially important for thwarting evolution of resistance in haplodiploid and parthenogenetic pests. Field-evolved resistance to Bt crops in only five pests during the last 14 years suggests that the refuge strategy has successfully delayed resistance, but the accumulation of resistant pests could accelerate.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1752-4571.2010.00129.x/pdf

Kruger, M.; van Rensburg, J.; Van Den Berg, J. 2011. Resistance to Bt Maize in *Busseola fusca* (*Lepidoptera: Noctuidae*) from Vaalharts, South Africa. *Environmental Entomology,* 40(2):477-483.

The first report of resistance of the maize stem borer [*Busseola fusca* (Fuller)] to Bt maize (MON810) was made in the Christiana area of South Africa during 2007. The objective of this study was to evaluate the status of resistance of other populations of *B. fusca* to Bt maize. One greenhouse and two laboratory studies were conducted. *B. fusca* populations were collected on Bt maize as well as the adjacent refugia (conventional maize and non-Bt maize) in the Vaalharts area, 50 km from the Christiana site. Control populations were collected from sites where Bt maize was not planted. In the greenhouse study 720 potted plants were each artificially infested with 10 neonate larvae of the F1-generation after the field collected populations were reared through to adults. Numbers of live larvae and larval mass per plant were determined at regular intervals over a 35-d period. Larvae of the Christiana conventional population (Bt-susceptible) on Bt maize (CHR08ConBt) and Bethal conventional population (Bt-susceptible) on Bt maize (BET08Con-Bt) did not survive on Bt maize for longer than 12 d. The populations collected from both Bt (VAA08Bt-Bt) maize and refuges (VAA08Ref-Bt) at Vaalharts were resistant and the subsequent generation of larvae completed their life cycle on Bt maize. Similar results were observed in the laboratory experiments. This study confirmed resistance of *B. fusca* to the Cry1Ab toxin (MON810). The geographical distribution of resistance was shown to include at least the Vaalharts area, in addition to the original report for the Christiana area. These observations that larvae collected from refugia at Vaalharts was resistant, show that the efficacy of the refuge strategy is compromised in this area because the contribution of refugia did not produce large enough numbers of susceptible individuals to mate with moths of which larvae survived inside Bt maize fields.

Artigo completo disponível em http://dspace.nwu.ac.za/bitstream/handle/10394/7429/ResistancetoBTmaizeEE40(2)s35eie.pdf?sequence=1

REV – Zhang, H.; Tian, W.; Zhao, J.; Jun, L.; Chunhui, Y.; Yihua, L.; Shuwen, Y.; Kongming, W.; *et al.* 2012. Diverse genetic basis of field-evolved resistance to Bt cotton in cotton bollworm from China. *Proceedings of the National Academy of Sciences,* vol. 109, issue 26, pp. 10275-10280.

Evolution of pest resistance reduces the efficacy of insecticidal proteins from Bacillus thuringiensis (Bt) used in sprays or in transgenic crops. Although several pests have evolved resistance to Bt crops in the field, information about the genetic basis of fieldevolved resistance to Bt crops has been limited. In particular, laboratory-selected resistance to Bt toxin Cry1Ac based on recessive mutations in a gene encoding a toxin-binding cadherin protein has been identified in three major cotton pests, but previous work has not determined if such mutations are associated with fieldselected resistance to Bt cotton. Here we show that the most common resistance alleles in field populations of cotton bollworm, Helicoverpa armigera, selected with Bt cotton in northern China, had recessive cadherin mutations, including the deletion mutation identified via laboratory selection. However, unlike all previously studied cadherin resistance alleles, one field-selected cadherin resistance allele conferred nonrecessive resistance. We also detected nonrecessive resistance that was not genetically linked with the cadherin locus. In field-selected populations, recessive cadherin alleles accounted for 75–84% of resistance alleles detected. However, most resistance alleles occurred in heterozygotes and 59–94% of resistant individuals carried at least one nonrecessive resistance allele. The results suggest that resistance management strategies must account for diverse resistance alleles in field-selected populations, including nonrecessive alleles.

Artigo completo disponível em http://www.pnas.org/content/109/26/10275.full.pdf

Tabashnik, B.; Gould, F. 2012. Delaying Corn Rootworm Resistance to Bt Corn. *Journal of Economic Entomology,* 105(3):767-776.

Transgenic crops producing *Bacillus thuringiensis* (Bt) toxins for insect control have been successful, but their efÞcacy is reduced when pests evolve resistance. To delay pest resistance to Bt crops, the U.S. Environmental Protection Agency (EPA) has required refuges of host plants that do not produce Bt toxins to promote survival of susceptible pests. Such refuges are expected to be most effective if the Bt plants deliver a dose of toxin high enough to kill nearly all hybrid progeny producedby matings between resistant and susceptible pests. In 2003, the EPA Þrst registered corn, *Zea mays* L., producing a Bt toxin (Cry3Bb1) that kills western corn rootworm, *Diabrotica virgifera virgifera* LeConte, one of the most economically important crop pests in the United States. The EPA requires minimum refuges of 20% for Cry3Bb1 corn and 5% for corn producing two Bt toxins active against corn rootworms.We conclude that the current refuge requirements are not adequate, because Bt corn hybrids active against corn rootworms do not meet the high-dose standard, and western corn rootworm has rapidly evolved resistance to Cry3Bb1 corn in the laboratory, greenhouse, and Þeld. Accordingly, we recommend increasing the minimum refuge for Bt corn targeting corn rootworms to 50% for plants producing one toxin active against these pests and to 20% for plants producing two toxins active against these pests. Increasing the minimum refuge percentage can help to delay pest resistance, encourage integrated pest management, and promote more sustainable crop protection.

http://www.ncbi.nlm.nih.gov/pubmed/22812111

Campagne, P.; Kruger, M.; Pasquet, R.; Le Ru, B.; Van den Berg, J. 2013. Dominant Inheritance of Field-Evolved Resistance to Bt Corn in *Busseola fusca*. *PLOS ONE,* 8(7): e69675. doi:10.1371/journal.pone.0069675.

Transgenic crops expressing Bacillus thuringiensis (*Bt*) toxins have been adopted worldwide, notably in developing countries. In spite of their success in controlling target pests while allowing a substantial reduction of insecticide use, the sustainable control of these pest populations is threatened by the evolution of resistance. The implementation of the “high dose/refuge” strategy for managing

insect resistance in transgenic crops aims at delaying the evolution of resistance to *Bt* crops in pest populations by promoting survival of susceptible insects. However, a crucial condition for the "high dose/refuge" strategy to be efficient is that the inheritance of resistance should be functionally recessive. *Busseola fusca* developed high levels of resistance to the *Bt* toxin *Cry 1Ab* expressed in *Bt* corn in South Africa. To test whether the inheritance of *B. fusca* resistance to the *Bt* toxin could be considered recessive we performed controlled crosses with this pest and evaluated its survival on *Bt* and non-*Bt* corn. Results show that resistance of *B. fusca* to *Bt* corn is dominant, which refutes the hypothesis of recessive inheritance. Survival on *Bt* corn was not lower than on non-*Bt* corn for both resistant larvae and the F_1 progeny from resistant × susceptible parents. Hence, resistance management strategies of *B. fusca* to *Bt* corn must address non-recessive resistance.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0069675

Outra estratégia adotada pelo setor das biotecnologias para maximizar a vida útil das tecnologias Bt – obtidas após investimentos de milhões de dólares – diz respeito ao desenvolvimento de variedades transgênicas "estaqueadas" ou "piramidadas", ou "empilhadas", em que dois ou mais transgenes são incorporados a uma mesma planta, por meio de cruzamentos convencionais envolvendo variedades transgênicas simples.

Essa estratégia, que inicialmente suscitou esperanças notáveis, rapidamente mostrou seus limites. Em tese, a resistência do inseto a uma das toxinas seria superada/complementada pela ação letal de outra toxina, com modo de ação diferente – impedindo a sobrevivência e a reprodução de indivíduos portadores de genomas insensíveis à tecnologia Bt. Atualmente a comunidade científica converge em considerar que plantas transgênicas piramidadas não impedem o desenvolvimento de populações de insetos insensíveis, em especial quando já existem na região populações resistentes a uma das toxinas sintetizadas no evento piramidado.

Zhao, J-Z.; Cao, J.; Collins, H.; Bates, S.; Roush, R.; Earle, E.; Shelton, A. 2005. Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. *PNAS*, 8426–8430, vol. 102, nº 24.

Transgenic plants expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) were grown on over 13 million ha in the United States and 22.4 million ha worldwide in 2004. Preventing or slowing the evolution of resistance by insects ("resistance management") is critical for the sustainable use of Bt crops. Plants containing two dissimilar Bt toxin genes in the same plant ("pyramided") have the potential to delay insect resistance. However, the advantage of pyramided Bt plants for resistance management may be compromised if they share similar toxins

with single-gene plants that are deployed simultaneously. We tested this hypothesis using a unique model system composed of broccoli plants transformed to express different Cry toxins (Cry1Ac, Cry1C, or both) and a synthetic population of the diamondback moth (*Plutella xylostella*) carrying genes for resistance to Cry1Ac and Cry1C at frequencies of _0.10 and 0.34, respectively. After 24–26 generations of selection in the greenhouse, the concurrent use of one- and two-gene plants resulted in control failure of both types of Bt plants. When only two-gene plants were used in the selection, no or few insects survived on one- or two-gene Bt plants, indicating that concurrent use of transgenic plants expressing a single and two Bt genes will select for resistance to two-gene plants more rapidly than the use of two-gene plants alone. The results of this experiment agree with the predictions of a Mendelian deterministic simulation model and have important implications for the regulation and deployment of pyramided Bt plants.

Artigo completo disponível em http://www.pnas.org/content/102/24/8426.full

Downes, S.; Parker, T.; Mahon, R. 2010. Incipient resistance of Helicoperva punctigera to the Cry2Ab Bt toxin in Bollgard II cotton. *PLOS ONE,* 5(9): e12567.

Combinations of dissimilar insecticidal proteins (“pyramids”) within transgenic plants are predicted to delay the evolution of pest resistance for significantly longer than crops expressing a single transgene. Field-evolved resistance to Bacillus thuringiensis (Bt) transgenic crops has been reported for first generation, single-toxin varieties and the Cry1 class of proteins. Our five year data set shows a significant exponential increase in the frequency of alleles conferring Cry2Ab resistance in Australian field populations of Helicoverpa punctigera since the adoption of a second generation, two-toxin Bt cotton expressing this insecticidal protein. Furthermore, the frequency of cry2Ab resistance alleles in populations from cropping areas is 8-fold higher than that found for populations from non-cropping regions. This report of field evolved resistance to a protein in a dual-toxin Bt-crop has precisely fulfilled the intended function of monitoring for resistance; namely, to provide an early warning of increases in frequencies that may lead to potential failures of the transgenic technology. Furthermore, it demonstrates that pyramids are not ‘bullet proof’ and that rapid evolution to Bt toxins in the Cry2 class is possible.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0012567

Brévault, T.; Heuberger, S.; Zhang, M.; Ellers-Kirk, K.; Ni, X.; Masson, L.; Li, X.; Tabashnik, B.; Carrière, Y. 2013. Potential shortfall of pyramided transgenic cotton for insect resistance management. *PNAS*, 5806–5811, vol. 110, nº 1.

To delay evolution of pest resistance to transgenic crops producing insecticidal proteins from *Bacillus thuringiensis* (Bt), the “pyramid” strategy uses plants that produce two or more toxins that kill the same pest. In the United States, this strategy has been adopted widely, with two-toxin Bt cotton replacing one-toxin Bt cotton. Although two-toxin plants are likely to be more durable than one-toxin plants, the extent of this advantage depends on several conditions. One key assumption favoring success of two-toxin plants is that they kill insects selected for resistance to one toxin, which is called “redundant killing.” Here we tested this assumption for a major pest, *Helicoverpa zea*, on transgenic cotton producing Bt toxins Cry1Ac and Cry2Ab. Selection with Cry1Ac increased survival on two-toxin cotton, which contradicts the assumption. The concentration of Cry1Ac and Cry2Ab declined during the growing season, which would tend to exacerbate this problem. Furthermore, analysis of results from 21 selection experiments with eight species of lepidopteran pests indicates that some cross-resistance typically occurs between Cry1A and Cry2A toxins. Incorporation of empirical data into simulation models shows that the observed deviations from ideal conditions could greatly reduce the benefits of the pyramid strategy for pests like *H. zea,* which have inherently low susceptibility to Bt toxins and have been exposed extensively to one of the toxins in the pyramid before two-toxin plants are adopted. For such pests, the pyramid strategy could be improved by incorporating empirical data on deviations from ideal assumptions about redundant killing and cross-resistance.

Artigo completo disponível em http://www.pnas.org/content/110/15/5806.full.pdf+html

Van den Berg, J.; Hilbeck, A.; Bøhn, T. 2013. Pest resistance to Cry1Ab Bt maize: Field resistance, contributing factors and lessons from South Africa. *Crop Protection,* 54, 154-160.

This paper documents the historical development of resistance of the African maize stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) to Bt maize (*Zea mays* L.). This pest was one of the first to evolve resistance to Bt maize expressing Cry1Ab protein. A time-line of events and contributing factors are presented, from the commencement of efficacy testing through to the present situation, where the Cry1Ab toxin has lost its efficacy against *B. fusca* at many localities throughout the maize producing region, and single-gene Bt maize events often require insecticide treatments for which farmers are compensated. Significant levels of pest survival on Bt maize was observed in the first season after commercial release in 1998 and confirmed seven years later. Reduced selection pressure on the target pest is the objective of insect resistance management (IRM), and strategies to accomplish this should receive highest priority. Where resistance is prevalent, the only viable options to reduce selection pressure are withdrawal of the product and/or enforcement of high-dose/refuge requirements. The latter action may however be of no value under conditions where resistance is prevalent, since the value of refugia to an IRM strategy may be compromised. Remedial actions taken in South Africa included the propagation and enforcement of refuge compliance followed by the release of pyramided maize hybrids in 2011. These pyramids combine Cry1A.105 and Cry2Ab2 toxin-producing transgenes, replacing the ineffective single-transgene. However, it remains uncertain if cross-resistance occurs between Cry1A.105/Cry2Ab2 and the closely related Cry1Ab toxin, and for how long this pyramided event will endure. Cultivation of Cry1Ab-expressing hybrids continues in areas where resistance levels have been confirmed to be high. In retrospect, this case provides lessons regarding IRM, not only in South Africa, but wherever Bt crops are being introduced.

http://www.sciencedirect.com/science/article/pii/S0261219413002093

De toda maneira, após alguns anos de uso comercial das plantas Bt, há consenso na comunidade científica sobre a necessidade de associar essa tecnologia a todo um conjunto de alternativas de manejo preexistentes, tais como uso de predadores naturais, uso complementar de agroquímicos, rotação de culturas, etc. Mantém-se o objetivo original de estender ao máximo a vida útil da tecnologia Bt, que por seu uso provoca a própria obsolescência. Ainda assim, pelo fato de as plantas Bt sintetizarem toxinas de modo contínuo e permanente ao longo do seu ciclo biológico, em todas suas células, alguns autores consideram as plantas Bt como sendo incompatíveis com o Manejo Integrado de Pragas (MIP).

Gray, M.; 2011. Relevance of Traditional Integrated Pest Management (IPM) Strategies for Commercial Corn Producers in a Transgenic Agroecosystem: A Bygone Era? *Journal of Agricultural and Food Chemistry,* 59 (11), pg. 5852-5858.

The use of transgenic Bt maize hybrids continues to increase significantly across the Corn Belt of the United States. In 2009, 59% of all maize planted in Illinois was characterized as a "stacked" gene variety. This is a 40% increase since 2006. Stacked hybrids typically express one Cry protein for corn rootworm control and one Cry protein for control of several lepidopteran pests; they also feature herbicide tolerance (to either glyphosate or glufosinate). Slightly more than 50 years has passed since Vernon Stern and his University of California entomology colleagues published (1959) their seminal paper on the integrated control concept, laying the foundation for modern pest management (IPM) programs. To assess the relevance of traditional IPM concepts within a transgenic agroecosystem, commercial maize producers were surveyed at a series of meetings in 2009 and 2010 regarding their perceptions on their use of Bt hybrids and resistance management. Special attention was devoted to two insect pests of corn, the European corn borer and the western corn rootworm. A high percentage of producers who participated in these meetings planted Bt hybrids in 2008 and 2009, 97 and 96.7%, respectively. Refuge compliance in 2008 and 2009, as mandated by the U.S. Environmental Protection Agency (EPA), was 82 and 75.7%, respectively, for those producers surveyed. A large majority of producers (79 and 73.3% in 2009 and 2010, respectively) revealed that they would, or had, used a Bt hybrid for corn rootworm (Diabrotica virgifera virgifera LeConte) or European corn borer (Ostrinia nubilalis Hübner) control even when anticipated densities were low. Currently, the EPA is evaluating the long-term use of seed blends (Bt and non-Bt) as a resistance management strategy. In 2010, a large percentage of producers, 80.4%, indicated they would be willing to use this approach. The current lack of integration of management tactics for insect pests of maize in the U.S. Corn Belt, due primarily to the escalating use of transgenic Bt hybrids, may eventually result in resistance evolution and/or other unforeseen consequences.

http://pubs.acs.org/doi/abs/10.1021/jf102673s

Birch, A.; Begg, G.; Squire, G. 2011. How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. *Journal of Experimental Botany*, page 1 of 11. doi:10.1093/jxb/err064

Drivers behind food security and crop protection issues are discussed in relation to food losses caused by pests. Pests globally consume food estimated to feed an additional one billion people. Key drivers include rapid human population increase, climate change, loss of beneficial on-farm biodiversity, reduction in per capita cropped land, water shortages, and EU pesticide withdrawals under policies relating to 91/414 EEC. IPM (Integrated Pest Management) will be compulsory for all EU agriculture by 2014 and is also being widely adopted globally. IPM offers a 'toolbox' of complementary crop- and region-specific crop protection solutions to address these rising pressures. IPM aims for more sustainable solutions by using complementary technologies. The applied research challenge now is to reduce selection pressure on single solution strategies, by creating additive/synergistic interactions between IPM components. IPM is compatible with organic, conventional, and GM cropping systems and is flexible, allowing regional fine-tuning. It reduces pests below economic thresholds utilizing key 'ecological services', particularly biocontrol. A recent global review demonstrates that IPM can reduce pesticide use and increase yields of most of the major crops studied. Landscape scale 'ecological engineering', together with genetic improvement of new crop varieties, will enhance the durability of pest-resistant cultivars (conventional and GM). IPM will also promote compatibility with semiochemicals, biopesticides, precision pest monitoring tools, and rapid diagnostics. These combined strategies are urgently needed and are best achieved via multi-disciplinary research, including complex spatio-temporal modelling at farm and landscape scales. Integrative and synergistic use of existing and new IPM technologies will help meet future food production needs more sustainably in developed and developing countries, in an era of reduced pesticide availability. Current IPM research gaps are identified and discussed.

Artigo completo disponível em http://jxb.oxfordjournals.org/content/early/2011/06/08/jxb.err064.full

2.2 Populações de ervas ruderais que desenvolvem resistência genética aos principais herbicidas usados em lavouras TH

Por motivos evolucionistas similares ao caso da perda de eficácia da tecnologia Bt ante o desenvolvimento de populações de insetos insensíveis às toxinas, produtores de plantas TH também enfrentam dificuldades de manejo das plantas ruderais nas suas lavouras.

De fato, o uso intensivo de algumas moléculas de produto ativo de herbicidas sistematicamente associadas ao cultivo de plantas TH, em especial as de glifosato, gerou uma pressão de seleção que fortaleceu populações de plantas ruderais naturalmente resistentes a esses herbicidas.

Os artigos listados nesse item ilustram esses casos de populações resistentes aos principais herbicidas sistematicamente associados ao cultivo de plantas TH (glifosato, glufosinato de amônio e, em fase de comercialização próxima, 2,4-D), salientando suas implicações em termos de dificuldades de manejo e combate. Hoje, há consenso na comunidade científica em considerar que essas populações representam uma ameaça severa à eficácia da tecnologia de tolerância a herbicidas, notadamente no caso do glifosato, por sua maior pressão de uso. Ainda assim, mecanismos similares de reação às pressões seletivas fatalmente levarão à emergência de plantas tolerantes aos demais princípios ativos, desde que utilizados da mesma forma massiva, em termos de abrangência e continuidade.

Owen, M.; Zelaya, I. 2005. Herbicide-resistant crops and weed resistance to herbicides. *Pest Manag.*, Sci. 61: 301-311.

The adoption of genetically modified (GM) crops has increased dramatically during the last 3 years, and currently over 52 million hectares of GM crops are planted world-wide. Approximately 41 million hectares of GM crops planted are herbicide-resistant crops, which includes an estimated 33.3million hectares of herbicide-resistant soybean. Herbicide-resistant maize, canola, cotton and soybean accounted for 77% of the GM crop hectares in 2001. However, sugarbeet, wheat, and as many as 14 other crops have transgenic herbicide-resistant cultivars that may be commercially available in the near future. There are many risks associated with the production of GM and herbicide-resistant crops, including problems with grain contamination, segregation and introgression of herbicide-resistant traits, marketplace acceptance and an increased reliance on herbicides for weed control. The latter issue is represented in the occurrence of weed population shifts, the evolution of herbicide-resistant weed populations and herbicide-resistant crops becoming volunteer weeds. Another issue is the ecological impact that simple weed management programs based on herbicide-resistant crops have on weed communities. Asiatic dayflower (*Commelina cumminus* L) common lambsquarters (*Chenopodium album* L) and wild buckwheat (*Polygonum convolvulus* L) are reported to be increasing in prominence in some agroecosystems due to the simple and significant selection pressure brought to bear by herbicideresistant crops and the concomitant use of the herbicide. Finally, evolution of herbicide-resistant weed populations attributable to the herbicide-resistant crop/herbicide program has been observed. Examples of herbicide-resistant weeds include populations of horseweed (*Conyza canadensis* (L) Cronq) resistant to *N*-(phosphonomethyl)glycine (glyphosate). An important question is whether or not these problems represent significant economic issues for future agriculture.

Artigo completo disponível em http://www.weeds.iastate.edu/weednews/2005/Herbicide-resistant%20crops%20and%20weed%20resistance%20to%20herbicides.pdf

Sandermann, H. 2006. Plant biotechnology: ecological case studies on herbicide resistance. *Trends in Plant Science,* 11 (7), 324–328.

The emerging field of molecular ecology aims to improve the ecological predictability of transgenic crop plants. The most widely cultivated lines are Roundup-Ready plants, which are genetically modified to be resistant to the broad-spectrum herbicide glyphosate. Recent publications demonstrate two ecological effects that were not anticipated: the widespread emergence of glyphosate-resistant weed biotypes and the formation of a metabolic herbicidal residue. Both effects appear to be due to the increased use of glyphosate rather than the genetic modification in the transgenic crop plant. With one prominent exception, opinions collected from the literature point towards a certain degree of resistance mismanagement and an inadequate testing of the ecological effects of extensive glyphosate use.

http://www.ncbi.nlm.nih.gov/pubmed/16781885

COM – Waltz, E. 2010. Glyphosate resistance threatens Roundup hegemony. *Nature Biotechnology,* 28, 537-538.

Sem resumo.

Artigo completo disponível em http://www.emilywaltz.com/NAS_-_June_2010.pdf

Gaines, T.; Zhang, W.; Wang, D.; Bukun, B.; Chisholm, S.; Shaner, D.; *et al.* 2010. Gene Amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proceedings of the National Academy of Sciences*, vol. 107 (3), 1029-1034.

The herbicide glyphosate became widely used in the United States and other parts of the world after the commercialization of glyphosate-resistant crops. These crops have constitutive overexpression of a glyphosate-insensitive form of the herbicide target site gene, 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*). Increased use of glyphosate over multiple years imposes selective genetic pressure on weed populations. We investigated recently discovered glyphosate-resistant *Amaranthus palmeri* populations from Georgia, in comparison with normally sensitive populations. EPSPS enzyme activity from resistant and susceptible plants was equally inhibited by glyphosate, which led us to use quantitative PCR to measure relative copy numbers of the *EPSPS* gene. Genomes of resistant plants contained from 5-fold to more than 160-fold more copies of the *EPSPS* gene than did genomes of susceptible plants. Quantitative RT-PCR on cDNA revealed that *EPSPS* expression was positively correlated with genomic *EPSPS* relative copy number. Immunoblot analyses showed that increased EPSPS protein level also correlated with *EPSPS* genomic copy number. *EPSPS* gene amplification was heritable, correlated with resistance in pseudo-F_2 populations, and is proposed to be the molecular basis of glyphosate resistance. FISH revealed that *EPSPS* genes were present on every chromosome and, therefore, gene amplification was likely not caused by unequal chromosome crossing over. This occurrence of gene amplification as an herbicide resistance mechanism in a naturally occurring weed population is particularly significant because it could threaten the sustainable use of glyphosate-resistant crop technology.

Artigo completo disponível em http://www.pnas.org/content/107/3/1029.full

Mortensen, D.; Egan, J.; Maxwell, B.; Ryan, M.; Smith, R. 2012. Navigating a Critical Juncture for Sustainable Weed Management. *BioScience,* vol. 62, nº 1, pp. 75-84.

Agricultural weed management has become entrenched in a single tactic—herbicide-resistant crops—and needs greater emphasis on integrated practices that are sustainable over the long term. In response to the outbreak of glyphosate-resistant weeds, the seed and agrichemical industries are developing crops that are genetically modified to have combined resistance to glyphosate and synthetic auxin herbicides. This technology will allow these herbicides to be used over vastly expanded areas and will likely create three interrelated challenges for sustainable weed management. First, crops with stacked herbicide resistance are likely to increase the severity of resistant weeds. Second, these crops will facilitate a significant increase in herbicide use, with potential negative consequences for environmental quality. Finally, the short-term fix provided by the new traits will encourage continued neglect of public research and extension in integrated weed management. Here, we discuss the risks to sustainable agriculture from the new resistant crops and present alternatives for research and policy.

Artigo completo disponível em http://bioscience.oxfordjournals.org/content/62/1/75.full

REV – Ward, S.; Webster, T.; Stecke, L. 2013. Palmer Amaranth (*Amaranthus palmeri*): A Review. *Weed Technology,* 27(1): 12-27.

In little over 20 yr, Palmer amaranth has risen from relative obscurity to its current status as one of the most widespread, troublesome, and economically damaging agronomic weeds in the southeastern U.S. Numerous factors have enabled Palmer amaranth to become such a dominant and difficult-to-control weed, including its rapid growth rate, high fecundity, genetic diversity, ability to tolerate adverse conditions, and its facility for evolving herbicide resistance. It is both a serious threat to several U.S. cropping systems and a fascinating model weed. In this paper, we review the growing body of literature on Palmer amaranth to summarize the current state of knowledge on the biology, agricultural impacts, and management of this weed, and we suggest future directions for research.

Artigo completo disponível em http://wssajournals.org/doi/full/10.1614/WT-D-12-00113.1

COM – Service, R. 2013. What Happens When Weed Killers Stop Killing? *Science,* 20, vol. 341 nº 6152 pp. 1329-1329.

Sem resumo.

http://www.sciencemag.org/content/341/6152/1329

COM - Nature Editorial, 2014. A growing problem. *Nature,* vol. 510, p. 187.

Sem resumo.

Artigo completo disponível em http://www.nature.com/news/a-growing-problem-1.15382

2.2.1 Resistência aos herbicidas à base de glifosato

REV – Nandula, V.; Reddy, K.; Duke, S.; Poston, D. 2005. Glyphosate-resistant weeds: Current status and future outlook. *Outlooks on Pest Management*, 16: 183–187.

Sem resumo.

Artigo completo disponível em http://afrsweb.usda.gov/SP2UserFiles/Place/64022000/Publications/Reddy/Nandula-GRW12.pdf

Vila-Aiub, M.; Vidal, A.; Balbi, M.; Gundel P.; Trucco, F.; Ghersa, C. 2007. Glyphosate-resistant weeds of South American cropping systems: an overview. *Pest Management Science*, 64, 366-371.

Herbicide resistance is an evolutionary event resulting from intense herbicide selection over genetically diverse weed populations. In South America, orchard, cereal and legume cropping systems show a strong dependence on glyphosate to control weeds. The goal of this report is to review the current knowledge on cases of evolved glyphosate-resistant weeds in South American agriculture. The first reports of glyphosate resistance include populations of highly diverse taxa (Lolium multiflorum Lam., Conyza bonariensis L., C. canadensis L.). In all instances, resistance evolution followed intense glyphosate use in fruit fields of Chile and Brazil. In fruit orchards from Colombia, Parthenium hysterophorus L. has shown the ability to withstand high glyphosate rates. The recent appearance of glyphosate-resistant Sorghum halepense L. and Euphorbia heterophylla L. in glyphosate-resistant soybean fields of Argentina and Brazil, respectively, is of major concern. The evolution of glyphosate resistance has clearly taken place in those agroecosystems where glyphosate exerts a strong and continuous selection pressure on weeds. The massive adoption of no-till practices together with the utilization of glyphosate-resistant soybean crops are factors encouraging increase in glyphosate use. This phenomenon has been more evident in Argentina and Brazil. The exclusive reliance on glyphosate as the main tool for weed management results in agroecosystems biologically more prone to glyphosate resistance evolution.

http://www.ncbi.nlm.nih.gov/pubmed/18161884

Vidal, A.; Trezzi, M.; Prado, R; Ruiz-Santaella, J.; Vila-Aiub, M. 2007. Glyphosate resistant biotypes of wild poinsettia (*Euphorbia heterophylla* L.) and its risk analysis on glyphosate-tolerant soybeans. *Journal of Food, Agriculture & Environment*, 5: 265-269.

The continuous use of a single herbicide for weed control can result in selection of biotypes resistant to that compound. Greenhouse experiments were conducted to assess the occurrence of wild poinsettia (*Euphorbia heterophylla*, EPHHL) resistant biotypes to glyphosate. Two suspected glyphosate- resistant biotypes from the northern part of Rio Grande do Sul, Brazil, were compared to known glyphosate-susceptible biotypes. Dose-response curves were used to compare the biotypes, with rates ranging from 0 to 450 g ha^{-1} in one experiment, and from 0 to 1200 g ha^{-1} in another. The resistance factor, calculated with the I_{50} data, indicated the resistant biotypes were about three times less sensitive to glyphosate than the susceptible biotypes. This is the first report of a glyphosate-resistant biotype in a weed species of major importance and distribution in Brazil. A risk analysis is discussed for the occurrence of glyphosate-resistant wild poinsettia in glyphosate-tolerant soybeans.

Artigo completo disponível em http://world-food.net/download/journals/2007-issue_2/e5.pdf

REV – Powles S. 2008. Evolved glyphosate-resistant weeds around the world: lesson to be learnt. *Pest Management Science,* 64, 360-365.

Glyphosate is the world's most important herbicide, with many uses that deliver effective and sustained control of a wide spectrum of unwanted (weedy) plant species. Until recently there were relatively few reports of weedy plant species evolving resistance to glyphosate. Since 1996, the advent and subsequent high adoption of tr~genic glyphosate-resistant crops in the Americas has meant unprecedented and often exclusive use of glyph,osate for weed controlover verylarge areas. Consequently, in regions oCthe USA where transgenic glyphosateresistant crops dominate, there are now evolved glyphosate-resistant populations of the economically damaging weed species *Ambrosia artemissifolia* L., *Atnbrosia trifida* L., *Atnaranthus paltneri* S Watson, *Amaranthus rudis'* JD Sauer, *Amaranthus tuberculatus* (Moq) JD Sauer and various *Conyza* and *Lolium* spp. Likewise, in areas' oftransgenic glyphosate-resistant crops in Argentina and Brazil, there are now evolved glyphosate-resistant populations of*Sorghum halepense* (L.) Pen and *Euphorbia hete-rophylla* L. respectively. As transgenic glyphosateresistant crops will remain very popular with producers, it is anticipated that glyphosate-resistant biotypes of other: prominent weed species will evolve over the next few years. Therefore, evolved glyphosate-resistant weeds are a major risk for the continued success of glyphosate and transgenic glyphosate-resistant crops. However, glyphosate-resistant weeds are not yet a problem in many parts ofthe world, and lessons can be learnt and actions taken, to achieve glyphosate sustainability. A major lesson is that maIntenance of diversity in weed management systems is crucial for glyphosate to be sustainable. Glyphosate is essential for present and future world food production, and action to secure its sustainability for future generations is a global imperative.

Artigo completo disponível em http://www.lpv.esalq.usp.br/lpv671/Review%20evolved%20glyphosate%20resistant%20weeds.pdf

REV - Binimelis, R.; Pengue, W.; Monterroso, I. 2009: Transgenic treadmill: Responses to the emergence and spread of glyphosate-resistant johnsongrass in Argentina. *Geoforum*, 40, 623-633.

The broad-spectrum herbicide glyphosate has become the largest-selling crop-protection product worldwide. The increased use of glyphosate is associated with the appearance of a growing number of tolerant or resistant weeds, with socio-environmental consequences apart from the loss of productivity. In 2002, a glyphosate-resistant biotype of johnsongrass (Sorghum halepense (L.)) appeared in Argentina and now covers at least 10,000 ha. This paper analyzes the driving forces behind the emergence and spread of this weed and also examines management responses and their implications. Preventive strategies against glyphosate-resistant johnsongrass fail because of the institutional setting. Reactive measures, however, transfer the risks to the society and the environment through the introduction of novel genetically modified crops that allow the use of yet more herbicide. This in turn reinforcesthe emergence of herbicide-resistant weeds, constituting a new phenomenon of intensification, the "transgenic treadmill".

http://stopogm.net/sites/stopogm.net/files/123456.pdf

Riar, D.; Norsworthy, J.; Johnson, D.; Scott, R.; Bagavathiannan, M. 2011. Glyphosate resistance in a Johnsongrass (*Sorghum halepense*) biotype from Arkansas. *Weed Science*, 59, 299-304. (1)

Johnsongrass is one of the most troublesome weeds of the world and is listed as a noxious weed in Arkansas. Reduced johnsongrass control with the recommended application rate of glyphosate (840 g ae ha21) was reported in a continuous soybean field near West Memphis, AR, in the fall of 2007. A greenhouse study was conducted (1) to confirm and characterize glyphosate resistance in the johnsongrass biotype from West Memphis and (2) to determine whether resistant and susceptible biotypes have differential glyphosate absorption or translocation. Dose–response studies revealed that the resistant biotype was five- to seven-fold less sensitive to glyphosate than the susceptible biotype. Glyphosate absorption was similar in resistant and susceptible biotypes at 72 h after treatment (HAT). However, the treated leaf of the resistant biotype retained 28 percentage points more absorbed 14C glyphosate compared to the susceptible biotype at 72 HAT. Additionally, the resistant biotype had less 14C glyphosate translocated to the aboveground tissue below the treated leaf and to roots compared to the susceptible biotype at 24 and 72 HAT. Reduced translocation and increased retention of glyphosate in treated leaves is a probable mechanism of resistance in this glyphosate-resistant johnsongrass biotype.

Artigo completo disponível em http://www.bioone.org/doi/pdf/10.1614/WS-D-10-00150.1

REV – Price, A.; Balkcom, K.; Culpepper, S.; Kelton, J.; Nichols, R.; Schomberg, H. 2011. Glyphosate-resistant Palmer amaranth: A threat to conservation tillage. *Journal of Soil and Water Conservation,* vol. 66(4) 265-275.

Conservation tillage reduces the physical movement of soil to the minimum required for crop establishment and production. When consistently practiced as a soil and crop management system, it greatly reduces soil erosion and is recognized for the potential to improve soil quality and water conservation and plant available water. Adoption of conservation tillage increased dramatically with the advent of transgenic, glyphosate-resistant crops that permitted in-season, over-the-top use of glyphosate (N-[phosphonomethyl] glycine), a broad-spectrum herbicide with very low mammalian toxicity and minimal potential for off-site movement in soil or water. Glyphosate-resistant crops are currently grown on approximately 70 million ha (173 million ac) worldwide. The United States has the most hectares (45 million ha [99 million ac]) of transgenic, glyphosate-resistant cultivars and the greatest number of hectares (46 million ha [114 million ac]) in conservation tillage. The practice of conservation tillage is now threatened by the emergence and rapid spread of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* [S.] Wats.), one of several amaranths commonly called pigweeds. First identified in Georgia, it now has been reported in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee. Another closely related dioecious amaranth, or pigweed, common waterhemp (*Amaranthus rudis* Sauer), has also developed resistance to glyphosate in Illinois, Iowa, Minnesota, and Missouri. Hundreds of thousands of conservation tillage hectares, some currently under USDA Natural Resources Conservation Service conservation program contracts, are at risk of being converted to higher-intensity tillage systems due to the inability to control these glyphosate-resistant *Amaranthus* species in conservation tillage systems using traditional technologies. The decline of conservation tillage is inevitable without the development and rapid adoption of integrated, effective weed control strategies. Traditional and alternative weed control strategies, such as the utilization of crop and herbicide rotation and integration of high residue cereal cover crops, are necessary in order to sustain conservation tillage practices.

Artigo completo disponível em http://www.ars.usda.gov/SP2UserFiles/Place/60100500/csr/ResearchPubs/price/price_11b.pdf

Gaines, T.; Cripps, A.; Powles, S. 2012. Evolved Resistance to Glyphosate in Junglerice (*Echinochloa colona*) from the Tropical Ord River Region in Australia. *Weed Technology* 26(3):480-484.

The objective of this study was to determine whether a junglerice population from the tropical Ord River region of northwest Australia was glyphosate resistant, and whether alternative herbicides labeled for junglerice control were still effective. Seed samples collected from the field site were initially screened with glyphosate in the glasshouse, and surviving individuals were self-pollinated for subsequent glyphosate dose-response studies. Glyphosate resistance was confirmed, as the suspected resistant population was found to be 8.6-fold more resistant to glyphosate than a susceptible population based on survival (LD_{50} of 3.72 kg ha^{-1}), and 5.6-fold more resistant based on biomass reduction (GR_{50} of 1.16 kg ha^{-1}). The glyphosate-resistant population was susceptible to label-recommended doses of all other herbicides assessed, including three acetyl-CoA carboxylase (ACC) –inhibiting herbicides (fluazifop-P, haloxyfop, and sethoxydim), two acetolactate synthase (ALS) –inhibiting herbicides (imazamox and sulfometuron), paraquat, and glufosinate. Glyphosate resistance has previously evolved in numerous species found in glyphosate-resistant cropping systems, no-till chemical fallow, fence line, and perennial crop situations. Here we report the evolution of glyphosate resistance in a cropping system that included annual tillage. The evolution of glyphosate resistance in junglerice from a tropical cropping system further demonstrates the need for improved glyphosate stewardship practices globally.

http://www.bioone.org/doi/abs/10.1614/WT-D-12-00029.1

2.2.2 Resistência aos herbicidas à base de 2,4-D

COM – Egan, J.; Maxwell, B.; Mortensen, D.; Ryan, M.; Smith, R. 2011. 2,4-Dichlorophenoxyacetic acid (2,4-D)-resistant crops and the potential for evolution of 2,4-D-resistant weeds. *PNAS,* 108(11): E37.

Sem resumo.

Artigo completo disponível em http://www.pnas.org/content/108/11/E37.full

Bernards, M.; Crespo, R.; Kruger, G.; Gaussoin, R.; Tranel, P. 2012. A Waterhemp (*Amaranthus tuberculatus*) Population Resistant to 2,4-D. *Weed Science*, 60 (3): 379 DOI: 10.1614/WS-D-11-00170.

A waterhemp population from a native-grass seed production field in Nebraska was no longer effectively controlled by 2,4-D. Seed was collected from the site, and dose-response studies were conducted to determine if this population was herbicide resistant. In the greenhouse, plants from the putative resistant and a susceptible waterhemp population were treated with 0, 18, 35, 70, 140, 280, 560, 1,120, or 2,240 g ae ha^{-1} 2,4-D. Visual injury estimates (I) were made 28 d after treatment (DAT), and plants were harvested and dry weights (GR) measured. The putative resistant population was approximately 10-fold more resistant to 2,4-D (RS ratio) than the susceptible population based on both I_{50} (50% visual injury) and GR_{50} (50% reduction in dry weight) values. The RS ratio increased to 19 and 111 as the data were extrapolated to I_{90} and GR_{90} estimates, respectively. GR_{50} doses of 995 g ha^{-1} for the resistant and 109 g ha^{-1} for the susceptible populations were estimated. A field dose-response study was conducted at the suspected resistant site with 2,4-D doses of 0, 140, 280, 560, 1,120, 2,240, 4,480, 8,960, 17,920, and 35,840 g ha^{-1}. At 28 DAT, visual injury estimates were 44% in plots treated with 35,840 g ha^{-1}. Some plants treated with the highest rate recovered and produced seed. Plants from the resistant and susceptible populations were also treated with 0, 9, 18, 35, 70, 140, 280, 560, or 1,120 g ae ha^{-1} dicamba in greenhouse bioassays. The 2,4-D resistant population was threefold less sensitive to dicamba based on I_{50} estimates but less than twofold less sensitive based on GR_{50} estimates. The synthetic auxins are the sixth mechanism-of-action herbicide group to which waterhemp has evolved resistance.

http://www.bioone.org/doi/abs/10.1614/WS-D-11-00170.1

2.2.3 Resistência aos herbicidas à base de Glufosinato de Amônio (GA)

Avila-Garcia, W.; Mallory-Smith, C. 2011. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. *Weed Science*, 59, 305-309.

Resistance to glufosinate has been confirmed in glyphosate-resistant Italian ryegrass populations collected in hazelnut orchards in Oregon. Dose–response, ammonia accumulation, and enzyme activity studies were conducted to test the sensitivity of three glyphosate-resistant and three susceptible Italian ryegrass populations to glufosinate. The glufosinate rates required to reduce the growth by 50% (GR_{50}) were 0.15, 0.18, and 0.21 for the control populations C1, C2, and C3, respectively, whereas for the resistant populations OR1, OR2, and OR3, the GR_{50} values were 0.49, 0.42, and 0.40 kg ai ha^{-1}, respectively, exhibiting an average resistance index of 2.4. The same trend was observed in ammonia accumulation studies between 48 and 96 h after glufosinate treatment where the susceptible populations accumulated on average two times more ammonia than the resistant populations. The glufosinate concentration required to reduce the glutamine synthetase enzyme activity by 50% (I_{50}) was not different for the resistant and susceptible populations. The I_{50}s ranged from 3.1 to 3.6 µM for the resistant populations and from 3.7 to 4.3 µM for the susceptible populations; therefore, an insensitive target site is not responsible for the glufosinate resistance.

Artigo completo disponível em http://wssajournals.org/doi/full/10.1614/WS-D-11-00012.1

2.3 Ineficiência das tecnologias de resistência a vírus

A tecnologia transgênica de resistência a vírus ainda é pouco desenvolvida e apresenta escassos resultados em termos de escala comercial[32]. Um dos principais motivos associa-se ao fato de que essa tecnologia recorre a mecanismos biológicos apenas parcialmente conhecidos/entendidos, submetidos a várias interações genéticas e epigenéticas susceptíveis de inativar o transgene ou impedir sua expressão total ou parcial, resultando na ineficácia de seus propósitos.

Nesses últimos anos, um dos mecanismos responsáveis pela ineficácia da tecnologia de resistência a vírus, obtida via transgenia, está sendo mais bem entendido. Trata-se do PTGS (*post-transducional*

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[32] No Brasil, esse tipo de tecnologia foi liberado comercialmente no caso do feijão 5.1, desenvolvido pela Embrapa. Com data de comercialização inicialmente prevista para 2013, a tecnologia ainda não está disponível para os produtores. Segundo a instituição que desenvolveu a tecnologia, esse atraso se deve a supostos problemas agronômicos inesperados (maior sensitividade a outros patógenos do feijão) e observados durante a fase de pré-comercialização – mas depois de autorização para uso comercial conferida pela CTNBio.

gene silencing[33]), mecanismo epigenético de silenciamento gênico que visa à destruição de todos os RNAm produzidos por determinados transgenes, bem como de todos os RNAm homólogos produzidos na célula. Ora, a presença permanente desses RNAm é indispensável para que a planta transgênica consiga exprimir a característica desejada – a resistência a vírus, nesse caso.

Pesquisadores identificaram que, ao "detectar" determinadas sequências genômicas, uma resposta local da planta envolvendo o PTGS se estende a todas as células, o que acaba inibindo o funcionamento da tecnologia.

Brault, V.; Pfeffer, S.; Erdinger, M.; Mutterer, J.; Ziegler-Graff, V. 2002. Virus-Induced Gene Silencing in Transgenic Plants Expressing the Minor Capsid Protein of *Beet western yellows virus. MPMI,* vol. 15, No. 8, pp. 799–807.

Transgenic *Nicotiana benthamiana* expressing the minor coat protein P74 of the phloem-limited *Beet western yellows virus* (BWYV) exhibited an unusual spatial pattern of posttranscriptional gene silencing (PTGS) when infected with BWYV or related viruses. Following infection, transgenic P74 and its mRNA accumulated to only low levels, 21 to 23 nucleotide RNAs homologous to the transgene appeared, and the transgene DNA underwent methylation. The infecting viral RNA, however, was not subject to significant silencing but multiplied readily and produced P74 in the phloem tissues, although the P74 encoded by the transgene disappeared from the phloem as well as the nonvascular tissues.

Artigo completo disponível em http://apsjournals.apsnet.org/doi/abs/10.1094/MPMI.2002.15.8.799

Lucioli, A.; Noris, E.; Brunetti, A.; Tavazza, R.; Ruzza, V.; *et al.* 2003. *Tomato Yellow Leaf Curl Sardinia Virus* Rep-Derived Resistance to Homologous and Heterologous Geminiviruses Occurs by Different Mechanisms and Is Overcome if Virus-Mediated Transgene Silencing Is Activated. *J. Virol.,* 77, 6785–6798.

The replication-associated protein (Rep) of geminiviruses is involved in several biological processes brought about by the presence of distinct functional domains. Recently, we have exploited the multifunctional character of the *Tomato yellow leaf curl Sardinia virus* (TYLCSV) Rep to develop a molecular interference strategy to impair TYLCSV infection. We showed that transgenic expression of its N-terminal 210 amino acids (Rep-210) confers resistance to the homologous virus by inhibiting viral transcription and replication. We have now used biochemical and transgenic approaches to carry out a fuller investigation of the molecular resistance mechanisms in transgenic plants expressing Rep-210. We show that Rep-210 confers resistance through two distinct molecular mechanisms, depending on the challenging virus. Resistance to the homologous virus is achieved by the ability of Rep-210 to tightly inhibit C1 gene transcription, while that to heterologous virus is due to the interacting property of the Rep-210 oligomerization domain. Furthermore, we present

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[33] Nem a qualidade e nem a quantidade dos RNAm produzidos são alteradas pelo mecanismo de PTGS, indicando esse mecanismo estar estritamente pós-transcricional.

evidence that in Rep-210-expressing plants, the duration of resistance is related to the ability of the challenging virus to shut off transgene expression by a posttranscriptional homology-dependent gene silencing mechanism. A model of Rep-210-mediated geminivirus resistance that takes transgene- and virus-mediated mechanisms into account is proposed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC156158/

Bian, X-Y.; Rasheed, M.; Seemanpillai, M.; Rezajan, M. 2006. Analysis of Silencing Escape of Tomato leaf curl virus: An Evaluation of the Role of DNA Methylation. *Mol. Plant Microbe Interact.,* 19, 614–624.

RNA silencing is a sequence-specific mechanism regulating gene expression and has been used successfully for antiviral defense against RNA viruses. Similar strategies to develop resistance against DNA containing *Tomato leaf curl virus* (TLCV) and some other geminiviruses have been unsuccessful. To analyze this silencing escape, we transformed tomato plants with a hairpin construct from the TLCV *C2* open reading frame (ORF). The transgenic plants showed a strong RNA silencing response, and following TLCV inoculation, their infection was delayed. However, the viral infection was not prevented and TLCV DNA accumulated to the levels found in nontransgenic plants. To determine the fate of a transgene carrying homology to the virus, we used transgenic plants carrying the TLCV *C4* gene, which induces a distinct phenotype. Upon TLCV infection, the phenotype was abolished and *C4* transcript disappeared. Concurrently, TLCV-specific small interfering RNAs were produced. In situ hybridization showed abundant levels of TLCV DNA in phloem cells of TLCV-infected *C4* transgenic plants. However, the *C4* transcripts were no longer detectable in nonvascular cells. Analysis of the transgene by methylation sequencing revealed a high level of de novo methylation of asymmetric cytosines in both the *C4* ORF and its 35S promoter. A high level of methylation also was found at both symmetric and asymmetric cytosines of the complementary-sense strand of TLCV double-stranded DNA. Given the previous finding that methylated geminiviral DNA is not competent for replication, we provide a model whereby TLCV evades host defense through a population of de novo synthesized unmethylated DNA.

Artigo completo disponível em http://apsjournals.apsnet.org/doi/abs/10.1094/MPMI-19-0614

Ribeiro, S.; Lohuis, H.; Goldbach, R.; Prins, M. 2007. Tomato Chlorotic Mottle Virus Is a Target of RNA Silencing but the Presence of Specific Short Interfering RNAs Does Not Guarantee Resistance in Transgenic Plants. *J. Virol.,* 81, 1563–1573.

Tomato chlorotic mottle virus (ToCMoV) is a begomovirus found widespread in tomato fields in Brazil.ToCMoV isolate BA-Se1 (ToCMoV–[BA-Se1]) was shown to trigger the plant RNA silencing surveillancein different host plants and, coinciding with a decrease in viral DNA levels, small interfering RNAs(siRNAs) specific to ToCMoV-[BA-Se1] accumulated in infected plants. Although not homogeneouslydistributed, the siRNA population in both infected *Nicotiana benthamiana* and tomato plantsrepresented the entire DNA-A and DNA-B genomes. We determined that in *N. benthamiana*, the primary targetscorresponded to the 5_ end of AC1 and the embedded AC4, the intergenic region and 5_ end of AV1 andoverlapping central part of AC5. Subsequently, transgenic *N. benthamiana* plants were generated that were preprogrammed to express double-stranded RNA corresponding to this most targeted portion of the virusgenome by using an intron-hairpin construct. These plants were shown to indeed produce ToCMoVspecificsiRNAs. When challenge inoculated, most transgenic lines showed significant delays in symptomdevelopment, and two lines had immune plants. Interestingly, the levels of transgene-produced siRNAs were similar in resistant and susceptible siblings of the same line. This indicates that, in contrast to RNA viruses, the mere presence of transgene siRNAs corresponding to DNA virus sequences does not guarantee virus resistance and that other factors may play a role in determining RNA-mediated resistance to DNA viruses.

Artigo completo disponível em http://jvi.asm.org/content/81/4/1563.full

COM – Lucioli, A.; Sallustio, D.; Barboni, D.; Berardi, A.; Papacchioli, V.; Tavazza, R.; Tavazza, M. 2008. A cautionary note on pathogen derived sequences. *Nat Biotechnol.,* v.26, p.617–619.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/18536679

Lafforgue, G.; Martinez, F.; Sardanyes, J.; Iglesia, F., Niu, Q-W.; Lin S-S., et al. 2011. Tempo and mode of plant RNA virus-escape from RNA interference-mediated resistance. *J Virol*, 85: 9686–95.

A biotechnological application of artificial microRNAs (amiRs) is the generation of plants that are resistant to virus infection. This resistance has proven to be highly effective and sequence specific. However, before these transgenic plants can be deployed in the field, it is important to evaluate the likelihood of the emergence of resistance-breaking mutants. Two issues are of particular interest: (i) whether such mutants can arise in nontransgenic plants that may act as reservoirs and (ii) whether a suboptimal expression level of the transgene, resulting in subinhibitory concentrations of the amiR, would favor the emergence of escape mutants. To address the first issue, we experimentally evolved independent lineages of *Turnip mosaic virus* (TuMV) (family Potyviridae) in fully susceptible wild-type Arabidopsis thaliana plants and then simulated the spillover of the evolving virus to fully resistant A. thaliana transgenic plants. To address the second issue, the evolution phase took place with transgenic plants that expressed the amiR at subinhibitory concentrations. Our results show that TuMV populations replicating in susceptible hosts accumulated resistance-breaking alleles that resulted in the overcoming of the resistance of fully resistant plants. The rate at which resistance was broken was 7 times higher for TuMV populations that experienced subinhibitory concentrations of the antiviral amiR. A molecular characterization of escape alleles showed that they all contained at least one nucleotide substitution in the target sequence, generally a transition of the G-to-A and C-to-U types, with many instances of convergent molecular evolution. To better understand the viral population dynamics taking place within each host, as well as to evaluate relevant population genetic parameters, we performed *in silico* simulations of the experiments. Together, our results contribute to the rational management of amiR-based antiviral resistance in plants.

Artigo completo disponível em http://jvi.asm.org/content/85/19/9686.full.pdf+html

Outros registros aplicados a riscos potenciais decorrentes do uso de tecnologias transgênicas de resistência a vírus apontam interações potenciais entre a proteína viral recombinante sintetizada em plantas transgênicas resistentes a vírus e outros vírus vegetais preexistentes. Isso poderia comprometer a eficiência de tais biotecnologias, além de gerar novos tipos de riscos, cuja avaliação se revela de enorme complexidade.

REV – Latham, J.; Wilson, A. 2008. Trans-complementation and Synergism in Plants: Implications for Viral Transgenes? *Molecular Plant Pathology, 9* (1): 85-103.

In plants, viral synergisms occur when one virus enhances infection by a distinct or unrelated virus. Such synergisms may be unidirectional or mutualistic but, in either case, synergism implies that protein(s) from one virus can enhance infection by another. A mechanistically related phenomenon is transcomplementation, in which a viral protein, usually expressed from a transgene, enhances or supports the infection of a virus from a distinct species. To gain an insight into the characteristics and limitations of these helper functions of individual viral genes, and to assess their effects on the plant–pathogen relationship, reports of successful synergism and transcomplementation were compiled from the peer-reviewed literature and combined with data from successful viral gene exchange experiments. Results from these experiments were tabulated to highlight the phylogenetic relationship between the helper and dependent viruses and, where possible, to identify the protein responsible for the altered infection process. The analysis of more than 150 publications, each containing one or more reports of successful exchanges, transcomplementation or synergism, revealed the following: (i) diverse viral traits can be enhanced by synergism and transcomplementation; these include the expansion of host range, acquisition of mechanical transmission, enhanced specific infectivity, enhanced cell-to-cell and long-distance movement, elevated or novel vector transmission, elevated viral titre and enhanced seed transmission; (ii) transcomplementation and synergism are mediated by many viral proteins, including inhibitors of gene silencing, replicases, coat proteins and movement proteins; (iii) although more frequent between closely related viruses, transcomplementation and synergism can occur between viruses that are phylogenetically highly divergent. As indicators of the interoperability of viral genes, these results are of general interest, but they can also be applied to the risk assessment of transgenic crops expressing viral proteins. In particular, they can contribute to the identification of potential hazards, and can be used to identify data gaps and limitations in predicting the likelihood of transgene-mediated transcomplementation.

http://www.ncbi.nlm.nih.gov/pubmed/18705887

Enfim, como no caso das plantas Bt e TH, é possível que a modificação genética de resistência a vírus termine por gerar perturbações ecológicas que resultem em danos agronômicos por insetos ou patógenos que até então não tinham expressão que os levasse a ser considerados pragas da lavoura[34]. Por exemplo, pesquisadores observaram que a presença do transgene de resistência a vírus num pepino transgênico fomentou a agressividade de determinados insetos pragas dessa planta. A maior “atratividade” da planta, para aqueles herbívoros, teria resultado em contaminação da lavoura com outro patógeno – uma bactéria letal para o pepino e transmitida por aquele inseto.

Sasu, M.; Ferrari, M.; Du, D.; Winsor, J.; Stephenson, A. 2009. Indirect costs of a nontarget pathogen mitigate the direct benefits of a virus-resistant transgene in wild Cucurbita. *Proc Natl Acad Sci USA,* 106(45): 19067-19071.

Virus-resistant transgenic squash are grown throughout the United States and much of Mexico and it is likely that the virus-resistant transgene (VRT) has been introduced to wild populations repeatedly. The evolutionary fate of any resistance gene in wild populations and its environmental impacts depend upon trade-offs between the costs and benefits of the resistance gene. In a 3-year field study using a wild gourd and transgenic and nontransgenic introgressives, we measured the effects of the transgene on fitness, on herbivory by cucumber beetles, on the incidence of mosaic viruses, and on the incidence of bacterial wilt disease (a fatal disease vectored by cucumber beetles). In each year, the first incidence of zucchini yellow mosaic virus occurred in mid-July and spread rapidly through the susceptible plants. We found that the transgenic plants had greater reproduction through both male and female function than the susceptible

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[34] Tais riscos são ilustrados no item 3.1 a seguir.

plants, indicating that the VRT has a direct fitness benefit for wild gourds under the conditions of our study. Moreover, the VRT had no effect on resistance to cucumber beetles or the incidence of wilt disease before the spread of the virus. However, as the virus spread through the fields, the cucumber beetles became increasingly concentrated upon the healthy (mostly transgenic) plants, which increased exposure to and the incidence of wilt disease on the transgenic plants. This indirect cost of the VRT (mediated by a nontarget herbivore and pathogen) mitigated the overall beneficial effect of the VRT on fitness.

Artigo completo disponível em http://www.pnas.org/content/106/45/19067.full

3 Problemas agronômicos não esperados associados ao cultivo de plantas transgênicas

3.1 Danos agronômicos por pragas secundárias e/ou potenciais em lavouras Bt

Em determinados casos – e notadamente quando ainda não houve desenvolvimento de populações de insetos insensíveis às proteínas Bt – agricultores que cultivam lavouras transgênicas “protegidas” pelas toxinas Cry podem vir a enfrentar danos significativos em função do ataque de outros insetos. A supressão das formas tradicionais de controle, bem como o vazio ecológico resultante da eliminação dos insetos alvo da tecnologia Bt, tem sido apontada como elemento de estímulo à emergência de pragas secundárias. Nessas condições, insetos que não causavam preocupações assumem tal característica de intensidade que podem provocar danos significativos, com impactos econômicos relevantes.

Isso se deve principalmente ao esvaziamento seletivo de determinados nichos ecológicos, que passam a ser ocupados por organismos competidores das espécies afetadas.

Ashouri, A.; Michaud, D.; Cloutier, C. 2001. Unexpected Effects of Different Potato Resistance Factors to the Colorado Potato Beetle (*Coleoptera: Chrysomelidae*) on the Potato Aphid (*Homoptera: Aphididae). Environmental Entomology*, vol. 30, no. 3, 524-532.

Improving the use of bioctechnological and classical plant resistance for herbivore pest control with less reliance on chemicals critically depends on predictable interactions with secondary pests. Performance of the potato aphid *Macrosiphum euphorbiae* (Thomas), a secondary pest of potato in eastern North America, was studied on potato, *Solanum tuberosum* L., lines with traits of potential resistance to primary pests. The lines tested were 'Newleaf', a transgenic 'Superior' cultivar expressing the *Bacillus thuringiensis* Berliner CryIIIA toxin, which is highly resistant to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); a transgenic 'Kennebec' cultivar expressing rice cystatin I, a protease inhibitor previously shown to inhibit cathepsin like digestive enzymes in the Colorado potato beetle; NYL 235–4, a potato derived by selective breeding following hybridization with *S. berthaulthii*, with a moderate density of glandular trichomes providing resistance to small insects by contact; and the commercial cultivars Superior and Kennebec used as controls. Transgenic Superior potatoes negatively affected *M. euphorbiae*'s growth and fecundity, in contrast with the OCI potato, which improved aphid performance. The flight incidence of young alatae of *M. euphorbiae* that completed development on transgenic Superior was significantly higher than in aphids from other potato lines. Aphid resistance in the 'NYL 235–4' line was complex and depended on aphid access being limited to leaves, which reduced survival and fecundity. However, when aphids had access to whole NYL 235–4 plants, aphid performance was restored, as they preferentially fed and reproduced on NYL 235–4 stems and apical buds of unfolding leaflets. The results illustrate that the performance of a secondary pest of potato can vary unpredictably, depending on the nature of the resistance factors involved in developing specific resistance to a primary pest.

http://www.bioone.org/doi/abs/10.1603/0046-225X-30.3.524?journalCode=enve

Men, X.; Ge, F.; Edwards, C.; Yardim, E. 2004. Influence of Pesticide Applications on Pest and Predatory Arthropods Associated with Transgenic Bt Cotton and Nontransgenic Cotton Plants. *Phytoparasitica,* 32(3):246-254.

The effects of pesticide applications on pests (aphids and acarid mites) and predators (ladybeetles and spiders) were investigated in transgenic *Bt* cotton and nontransgenic cotton agroecosystems in 1999, 2000 and 2001. Transgenic cotton did not cause changes in populations of acarids and did not reduce numbers of predators considerably; its effects on aphids were inconsistent. Although insecticides were not applied against the main pest . cotton bollworm . on transgenic cotton, the total number of insecticide applications in 3 years was no less than the total applied on nontransgenic cotton, because additional applications were required against sucking pests on transgenic *Bt* cotton. Pesticide applications decreased numbers of aphids, acarids and predatory spiders significantly on both transgenic and nontransgenic cottons. The results suggest that the use of *Bt* cotton should be evaluated carefully in China.

Artigo completo disponível em http://www.biosci.ohio-state.edu/~soilecol/Full%20articles/Ecology%20and%20Ecotoxicology/2004/Influence%20of%20pesticide%20applications.pdf

Men, X.; Ge, F.; Edwards, C.; Yardim, E. 2005. The influence of pesticide applications on Helicoverpa armigera Hübner and sucking pests in transgenic Bt cotton and non-transgenic cotton in China. *Crop Protection,* 24 (4): 319-324.

Effects of pesticide applications, based on an IPM program on cotton bollworm, *Helicoverpa armigera* Hübner, cotton mirids and cotton leafhoppers, were evaluated in transgenic *Bt*-cotton and non-transgenic cotton agroecosystems between 1999 and 2001 in China. Differences in pest populations between cotton varieties were also compared. In 1999 and 2000, bollworm populations on non-transgenic cotton were larger than those on transgenic *Bt*-cotton. In *Bt*-cotton fields, the numbers of fourth-generation bollworms were greater than those of in the second and the third generations over all 3 years of study. Leafhopper populations on *Bt*-cotton were consistently larger than those on non-transgenic cotton during the 3 years of study. Although the use of transgenic *Bt*-cotton decreased the need for insecticide applications against cotton bollworm, this relaxation from pesticide applications could cause increased populations of sucking insects, which could require additional insecticide applications.

http://www.sciencedirect.com/science/article/pii/S0261219404002182

COM – Pearson, H. 2006. Transgenic cotton drives insect boom. *Nature*. doi:10.1038/news060724-5.

Sem resumo.

Artigo completo disponível em http://www.nature.com/news/2006/060724/full/news060724-5.html

Catangui, M.; Berg, R. 2006. Western bean cutworm, *Striacosta albicosta* (Smith) *(Lepidoptera: Noctuidae*), as a potential pest of transgenic Cry1Ab Bacillus thuringiensis corn hybrids in South Dakota. *Environmental Entomology,* 35: 1439-1452.

Injuries caused by the western bean cutworm, *Striacosta albicosta* (Smith), on transgenic Cry1Ab *Bacillus thuringiensis* (*Bt*) corn hybrids were documented and quantified. The western bean cutworm is an emerging or potential pest of transgenic *Bt* corn in South Dakota. The proportion of ears infested with western bean cutworm larvae in the Cry1Ab *Bt* corn hybrids were 18-20, 38-70, and 0-34% in 2000, 2003, and 2004, respectively. The Cry1Ab *Bt* corn hybrids were almost completely free of European corn borer infestations. Untreated conventional corn hybrids were less infested with western bean cutworm larvae but more infested with European corn borer larvae. The proportion of ears infested with European corn borer larvae alone were 33, 58-80, and 8-25% in 2000, 2003, and 2004, respectively. Infestations with western bean cutworm alone were 28, 8-28, and 13-19%, respectively. Proportion of ears simultaneously infested with both western bean cutworm and European corn borer larvae were much lower than single infestations by either species alone, indicating niche overlap and competition. Simultaneous infestations by the two species on untreated conventional corn hybrids were only 8, 0-18, and 0-1% in 2000, 2003, and 2004. The corn grains harvested from injured ears were also analyzed for fumonisin and aflatoxin through quantitative enzyme-linked immunosorbent assays. More mycotoxins were found in 2003 when the levels of insect infestation in the corn ears were higher than in 2004. Results from this study underscore the need to investigate other emerging or potential arthropod pests of transgenic *Bt* corn hybrids in addition to the western bean cutworm.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/webfm/plataforma/westernbeancutwormstriacostaalbicostasmith.pdf

Wang, S.; Just, D. 2008. Pinstrup-Andersen P. Bt-cotton and secondary pests. *Int. J. Biotechnology,* 10(2/3): 113–121.

Bt-cotton seed has been effective to control the damage of bollworm in Chinese cotton production since 1999, reducing the need for pesticides and increasing incomes of Chinese farmers. Field data collected in 2004 indicates that these benefits have been eroded by increasing the use of pesticides aimed to control secondary pests. The combination of Bt-cotton seed and other forms of biological pest control may help farmers regain the economic and environmental benefits of previous years. Failure to find a solution, may lead to the discontinuation of the use of Bt-cotton seed in China and elsewhere.

http://www.inderscience.com/info/inarticle.php?artid=18348

Cloutier, C.; Boudreault, S.; Michaud, D. 2008. Impact of Colorado potato beetle resistant potatoes on non-target arthropods: a meta-analysis of factors potentially involved in the failure of a Bt transgenic plant. *Cahiers Agricultures,* 17 (4): 388-394.

The relatively high specificity of transgenic plants based on Cry toxins of Bacillus thuringiensis (Bt) implies the possibility of upward agroecosystemic cascades toward new equilibria among arthropods associating with cultivated plants. We examine the hypothesis that exclusion of the Colorado potato beetle from potato expressing the Cry3a toxin increases the abundance of non-target herbivores, which indirectly favours the abundance of herbivore-dependent predators and omnivores foraging on agricultural plants. We examined the impact of Bt potato on non-target arthropod taxa, based on impact studies conducted during development of the Newleaf® Bt potato in North America. Of 32 field tests comparing Bt potato to non-transgenic controls, 14 (42%) revealed a significant, positive effect on the abundance of sucking insects (aphids, leafhoppers, mirids, thrips). Among 72 tests on generalist predators that were simultaneously monitored, 14 (~20%) also revealed significant positive effects. Such positive effects on predators can best be explained by their abundance being increased as a result of greater productivity due to overabundance of sucking insect prey, which are selectively favoured by the high specificity of the Cry3a Bt toxin. Our results support the idea that development of the Bt potato may have been hampered in part by its positive effects on sucking insect pests, and underline the importance of conserving the natural enemies of secondary pests that are indirectly favoured.

Artigo completo disponível em http://www.jle.com/en/revues/agr/e-docs/impact_de_pommes_de_terre_resistantes_au_doryphore_sur_les_arthropodes_non_vises_une_meta_analyse_des_facteurs_possiblement_e_278639/article.phtml?tab=texte

Dorhout, D.; Rice, M. 2010. Intraguild competition and enhanced survival of western bean cutworm *(Lepidoptera: Noctuidae*) on transgenic Cry1Ab (MON810) *Bacillus thuringiensis* corn. *Journal of Economic Entomology,* 103: 54–62.

The effect of genetically modified corn (event MON810, YieldGard Corn Borer) expressing the Bacillus thuringiensis sp. kurstaki (Berliner) (Bt) endotoxin, Cry1Ab, on the survival of western bean cutworm, Striacosta albicosta (Smith), larvae was examined during intraguild competition studies with either European corn borer, Ostrinia nubilalis (Hübner), or corn earworm, Helicoverpa zea (Boddie), larvae. Competition scenarios were constructed by using either a laboratory or field competition arena containing one of five different diets and one of 13 different larval size-by-species scenarios. The survival of western bean cutworms competing with corn earworms in the laboratory arenas on either a meridic diet or isoline corn silk diet was significantly lower (P < or = 0.01) than the controls in 13 out of 14 competition scenarios and larval survival was frequently zero. In contrast, the survival of western bean cutworm competing with corn earworm on a Cry1Ab-MON810 corn silk diet was significant higher (P < or = 0.01) than the controls in four out of six competition scenarios. The results observed in the three way competitions involving the addition of European corn borers generally did not alter the outcomes observed in the western bean cutworm and corn earworm only two-way competitions. These data suggest that Cry1Ab-MON810 corn may confer a competitive advantage to western bean cutworm larvae during intraguild competition, particularly from corn earworms, and that western bean cutworms become equal competitors only when they are of equal or larger size and the diet is Cry1Ab-MON810 corn.

http://www.ncbi.nlm.nih.gov/pubmed/20214368

Lu, Y.; Wu, K.; Jiang, Y.; Xia, B.; *et al*. 2010. Mirid Bug Outbreaks in Multiple Crops Correlated with Wide-Scale Adoption of Bt Cotton in China. *Science,* vol. 328 nº 5982 pp. 1151-1154.

Long-term ecological effects of transgenic *Bacillus thuringiensis* (Bt) crops on nontarget pests have received limited attention, more so in diverse small holder–based cropping systems of the developing world. Field trials conducted over 10 years in northern China show that mirid bugs (Heteroptera: Miridae) have progressively increased population sizes and acquired pest status in cotton and multiple other crops, in association with a regional increase in Bt cotton adoption. More specifically, our analyses show that Bt cotton has become a source of mirid bugs and that their population increases are related to drops in insecticide use in this crop. Hence, alterations of pest management regimes in Bt cotton could be responsible for the appearance and subsequent spread of nontarget pests at an agro-landscape level.

Artigo completo disponível em http://www.sciencemag.org/content/328/5982/1151.full

Zhao, J.; Ho, P.; Azadi, H. 2011. Benefits of Bt cotton counterbalanced by secondary pests? Perceptions of ecological change in China. *Environmental Monitoring and Assessment*, volume 173, Issue 1-4, pp 985-994.

In the past, scientific research has predicted a decrease in the effectiveness of Bt cotton due to the rise of secondary and other sucking pests. It is suspected that once the primary pest is brought under control, secondary pests have a chance to emerge due to the lower pesticide applications in Bt cotton cultivars. Studies on this phenomenon are scarce. This article furnishes empirical evidence that farmers in China perceive a substantial increase in secondary pests after the introduction of Bt cotton. The research is based on a survey of 1,000 randomly selected farm households in five provinces in China. We found that the reduction in pesticide use in Bt cotton cultivars is significantly lower than that reported in research elsewhere. This is consistent with the hypothesis suggested by recent studies that more pesticide sprayings are needed over time to control emerging secondary pests, such as aphids, spider mites, and lygus bugs. Apart from farmers' perceptions of secondary pests, we also assessed their basic knowledge of Bt cotton and their perceptions of Bt cotton in terms of its strengths and shortcomings (e.g., effectiveness, productivity, price, and pesticide use) in comparison with non-transgenic cotton.

http://www.ncbi.nlm.nih.gov/pubmed/20437270

Hagenbucher, S.; Wackers, F.; Wettstein, F.; Olson, D.; Ruberson, J.; Romeis J. 2013. Pest tradeoffs in technology: reduced damage by caterpillars in Bt cotton benefits aphids. *Proceedings of the Royal Society B,* 20130042.

The rapid adoption of genetically engineered (GE) plants that express insecticidal Cry proteins derived from Bacillus thuringiensis (Bt) has raised concerns about their potential impact on non-target organisms. This includes the possibility that non-target herbivores develop into pests. Although studies have now reported increased populations of non-target herbivores in Bt cotton, the underlying mechanisms are not fully understood. We propose that lack of herbivore-induced secondary metabolites in Bt cotton represents a mechanism that benefits non-target herbivores.We showthat, because of effective suppression of Bt-sensitive lepidopteran herbivores, Bt cotton contains reduced levels of induced terpenoids.We also showthat changes in the overall level of these defensive secondary metabolites are associated with improved performance of a Bt-insensitive herbivore, the cotton aphid, under glasshouse conditions. These effects, however,were not as clearly evident under field conditions as aphid populations were not correlated with the amount of terpenoids measured in the plants. Nevertheless, increased aphid numbers were visible in Bt cotton compared with non-Bt cotton on some sampling dates. Identification of this mechanism increases our understanding of how insect-resistant crops impact herbivore communities and helps underpin the sustainable use of GE varieties.

Artigo completo disponível em http://rspb.royalsocietypublishing.org/content/280/1758/20130042.full

Bortolotto, O.; Silva, G.; Bueno, A.; Pomari, A.; Martinelli, S.; Head, G.; Carvalho, R.; Barbosa, G. 2014.[35]Development and reproduction of Spodoptera eridania (Lepidoptera: Noctuidae) and its egg parasitoid Telenomus remus (Hymenoptera: Platygastridae) on the genetically modified soybean (Bt) MON 87701×MON 89788. *Bull Entomol Res,* 104(6):724-30. doi: 10.1017/S0007485314000546. Epub 2014 Sep 24.

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[35] Um corrigendum, que trata de minimizar os riscos associados ao uso deste evento (de soja Bt) e de reduzir a possibilidade de efeitos não intencionais da transgenia (explicitados no item 2 da Parte 1 dessa publicação), foi publicado pelos mesmos autores e está disponível em http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=9500235&fileId=S0007485314000881.

Genetically modified crops with insect resistance genes from Bacillus thuringiensis Berliner (Bt-plants) are increasingly being cultivated worldwide. Therefore, it is critical to improve our knowledge of their direct or indirect impact not only on target pests but also on non-target arthropods. Hence, this study evaluates comparative leaf consumption and performance of Spodoptera eridania (Cramer), a species that is tolerant of the Cry1Ac protein, fed with Bt soybean, MON 87701×MON 89788 or its non-Bt isoline. We also assessed the comparative performance of the egg parasitoid Telenomus remus Nixon on eggs of S. eridania produced from individuals that fed on these two soybean isolines as larvae. Results showed that Bt soybean reduced by 2 days larval development and increased by 3 days adult male longevity. Therefore, we conclude that the effect of Bt soybean MON 87701×MON 89788 on S. eridania development and reproduction is small, and favorable to pest development. These differences are less likely to directly result from the toxin presence but indirectly from unintended changes in plant characteristics caused by the insertion of the transgene. Our results should be viewed as an alert that S. eridania populations may increase in Bt soybeans, but on the other hand, no adverse effects of this technology were observed for the egg parasitoid T. remus which can help to prevent S. eridania outbreaks on these crops.

http://www.ncbi.nlm.nih.gov/pubmed/25248849

Em paralelo, o uso da tecnologia Bt em larga escala pode reduzir drasticamente populações de determinadas espécies alvo e não alvo, agravando os desequilíbrios ecológicos existentes nos agroecossistemas. Ora, algumas dessas espécies são por vezes inimigos naturais (ou competidores) de outras, potencialmente pragas dos mesmos cultivos. Nessas circunstancias, a eliminação de alguns insetos promove explosões populacionais de outros – não sensíveis ao Bt. A literatura científica tem registrado emergência de novas pragas em lavouras de milho Bt, supostamente estimuladas pela redução populacional de outras, que controlavam as primeiras e que tiveram suas populações reduzidas por ser alvo do Bt[36].

O'Rourke, P.; Hutchinson, W. 2000. First report of the western bean cutworm, *Richia albicosta* (Smith) (*Lepidoptera: Noctuidae*), in Minnesota corn. *J. Agric. Urban. Entomol.,* 17: 213-217.

The incidence of *R. albicosta* in Bt ('GH-0937', expressing a protein toxin Cry1Ab from *Bacillus thuringiensis* var. *kurstaki*) and non-Bt sweetcorn hybrids ('Bonus') was investigated during 1999 in 5 locations in southern Minnesota, USA. Larvae of *R. albicosta*, which were observed feeding on kernel tissues of both Bt and non-Bt sweetcorn hybrids, were collected from four of the five locations surveyed. Across locations, the difference in mean density of *R. albicosta* in Bt sweetcorn (0.0080 larvae per ear) and non-Bt sweetcorn (0.0050 larvae per ear) was not significant. The low-density infestation suggests that Cry1Ab does not provide significant control against *R. albicosta*, which is known to

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[36] No Brasil essa cadeia de efeitos ecológicos é apontada como responsável por grandes prejuízos econômicos enfrentados pelos produtores de soja e algodão (avaliados em milhões de reais para o Estado da Bahia, anos 2012-2013). De fato, a eliminação de grandes populações de *Spodoptera sp.* (predador natural da praga *Helicoverpa armigera*) teria conduzido a uma explosão populacional de *H. armigera*, nova espécie de praga da soja e do algodão, entre outros cultivos. Não foram encontrados artigos a esse respeito em periódicos da literatura científica.

have differential susceptibility to *B. thuringiensis* toxins. This is claimed to be the first documented occurrence of *R. albicosta* in Minnesota.

http://cabdirect.org/abstracts/20013070327.html;jsessionid=FA940A16B8CB269BB6FCBAB190AE133C

Tooker, J.; Fleischer, S. 2010. First report of western bean cutworm (*Striacosta albicosta*) in Pennsylvania. Online. *Crop Management*, doi:10.1094/CM-2010-0616-01-RS.

These results demonstrate that western bean cutworm moths were present in Pennsylvania in 2009, and the distribution and quality of the specimens suggest that populations have established. The data also suggest that universal traps work well for detection of low-level populations at least as well as milk jug traps.

Artigo completo disponível em http://ento.psu.edu/publications/wbc-pa

Michel, A.; Krupke, C.; Baute, T.; Difonzo, C. 2010. Ecology and Management of the Western Bean Cutworm (Lepidoptera:Noctuidae) in Cornand Dry Beans. *Journal of Integrated Pest Management*, 1(1): 2010; DOI:10.1603/IPM10003.

The western bean cutworm, *Striacosta albicosta* (Smith) (Lepidoptera:Noctuidae),is a native North American pest that feeds mainly on corn and dry beans. The historical geographic range of the western bean cutworm coveredthe western Great Plains states, including Colorado, Nebraska, and Wyoming. Since 1999, the geographic range of the western bean cutworm hasrapidly expanded east ward across the United States Corn Belt, causing significant and economic damage to corn and dry beans in parts of this region. This expansion has led to a resurgence of interesting this pest, particularly in areas where it has most recently caused damage. We summarize the ecology and biology of western bean cutworm and discuss options for scouting and management, with an emphasis in the expanded geographical range.

Artigo completo disponível em http://extension.entm.purdue.edu/fieldcropsipm/pubs/WBC_JIPM_Krupke.pdf

3.2 Danos agronômicos pelo uso intensivo dos herbicidas associados às plantas TH

A tecnologia de tolerância a herbicidas (TH) é dependente do uso de um determinado herbicida – em geral sistêmico – que será aplicado em vários momentos do ciclo de produção, de forma isolada ou combinada com outro(s) herbicida(s) que também não cause(m) danos letais àquela planta. Tal facilidade de operar aplicações independentemente do ciclo da cultura simplificou profundamente o manejo agrícola das principais lavouras, ao mesmo tempo em que expandiu drasticamente as vendas de agroquímicos associados às variedades transgênicas do tipo TH.

Além dos impactos para a saúde e o meio ambiente, decorrentes da expansão no uso dos mesmos venenos, a tecnologia demonstrou capacidade de gerar perturbações na microbiota do solo e nas trocas de nutrientes solo-planta, diminuindo capacidade produtiva da lavoura, ocasionando ainda danos agronômicos a lavouras situadas em áreas adjacentes ou nos mesmos locais, em safras subsequentes.

3.2.1 Impactos negativos do glifosato sobre a produtividade de plantas TH

Muitos documentos disponíveis na literatura científica apontam que herbicidas à base de glifosato enfraquecem fisiologicamente as plantas (destaque para a soja), afetam comunidades de microbiota do solo que contribuem ao bom desempenho agronômico dos cultivos e criam desequilíbrios nos bancos de sementes e relações populacionais a eles associados.

Ismail, B.; Kader, A.; Omar, O. 1995. Effects of Glyphosate on Cellulose Decomposition in Two Soils. *Folia Microbiol.*, 40 (5), 499-502.

Glyphosate with an equivalent concentration of either 0, 2.16 or 8.64 kg/hm² was sprayed on to cellulosic materials before burying in two soil types; peat (soil I) and sandy clay loam (soil II). Alternatively the soils were sprayed with 0, 20 or 150 ppm of the herbicide before burying the cellulosic material either immediately or after preincubation for 4 weeks. In soil I, the increase in glyphosate concentrations substantially reduced the decomposition of cellulosic material regardless of the method of application employed. Glyphosate at 8.64 kg/hm² reduced the mass loss of the treated substrate by 83 %. However, cellulose decomposition in soil preincubated for 4 weeks before burying was affected almost to the same extent as the untreated control. Glyphosate stimulated cellulose decomposition when substrates were buried in soil II. Mass loss in soil treated with 150 ppm increased by about 100 % while when glyphosate was sprayed directly to the substrate (at 8.64 kg/hm²), the loss was about 25 %.

http://link.springer.com/article/10.1007/BF02814731

King, C.; Purcell, L.; Vories, E. 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. *Agronomy Journal,* 93 (1), 179-186.

Glyphosate [*N*-(phosphonomethyl)glycine] inhibits 5-enolpyruvylshikimate-3-phosphate synthase, EC 2.5.1.19 (EPSPS), thereby blocking aromatic amino acid synthesis. While glyphosate-tolerant (GT) soybean [*Glycine max* (L.) Merr.] contains resistant EPSPS, the N_2-fixing symbiont in soybean root nodules, *Bradyrhizobium japonicum*, does not contain a resistant enzyme, and glyphosate spray to GT soybean may interfere with the symbiotic relationship. Glyphosate-tolerant soybean was treated with glyphosate at several different stages of development to evaluate N_2 fixation, growth, and yield in a series of greenhouse, growth chamber, and field experiments. Early applications of glyphosate generally delayed N_2 fixation and decreased biomass and N accumulation in the cultivar Terral TV5866RR (TV5866RR) harvested at 19 d after emergence (DAE), but plants had recovered by 40 DAE. The biomass and N content of GT soybean were also decreased by glyphosate in plants that were grown with available soil N. There were differences in sensitivity to glyphosate among GT cultivars, with biomass decreases in response to glyphosate ranging from 0 to 30% at 40 DAE for the most tolerant and sensitive cultivars that were evaluated. In growth chamber studies, N_2 fixation was more sensitive to water deficits in glyphosate-treated plants. In field studies, there was no measured effect of glyphosate on GT soybean at Fayetteville, AR where there was adequate soil water throughout the growing season. However, glyphosate tended to decrease biomass and seed yields under conditions of limited soil water at Keiser, AR.

https://www.agronomy.org/publications/aj/abstracts/93/1/179

Reddy, K.; Zablotowicz, R. 2003. Glyphosate resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Science,* 51 (4): 496-502.

A field study was conducted during 2000 and 2001 at Stoneville, MS, to determine the effects of isopropylamine, trimethylsulfonium (Tms), diammonium, and aminomethanamide dihydrogen tetraoxosulfate (Adt) salt formulations of glyphosate on weed control, growth, chlorophyll content, nodulation, nitrogen content, and grain yield in glyphosate-resistant soybean and to assess potential glyphosate accumulation in soybean nodules. Glyphosate-Tms and glyphosate-Adt injured soybean, and visible injury ranged from 29 to 38% 2 d after late postemergence (LPOST) application; however, soybean recovered by 14 d. Glyphosate formulations had no effect on chlorophyll content, root and shoot dry weight, or nodule number but reduced nodule biomass by 21 to 28% 14 d LPOST. Glyphosate levels in nodules from treated plants ranged from 39 to 147 ng g21 (dry weight), and leghemoglobin content was reduced by as much as 10%. Control of five predominant weed species 14d after LPOST was . 83% with one application and . 96% with two applications regardless of the glyphosate salts used. Soybean yields were generally higher with two applications than with one application regardless of glyphosate formulation. These results indicate that soybean injury and inhibition of nodule development with certain glyphosate formulations can occur, but soybean has the potential to recover from glyphosate stress.

Artigo completo disponível em http://naldc.nal.usda.gov/download/48637/PDF

Huber, D.; Cheng, M.; Winsor, B. 2005. Association of severe Corynespora root rot of soybean with glyphosate-killed giant ragweed. *Phytopathology*, 95, S45.

The soilborne pathogen Corynespora cassiicola was the predominant fungus isolated from severely stunted soybeans adjacent to glyphosate-killed giant ragweed plants (Ambrosia trifida) in Indiana fields. Soybeans adjacent to glyphosate-killed ragweed exhibited dark-brown to black lesions on 90–95% of their roots and hypocotyls. In contrast, soybeans that were not adjacent to dead Ambrosia trifida, or that were adjacent to living ragweed plants, exhibited only 5–10% root rot; and a number of different soilborne fungi in addition to Corynespora were isolated from these roots. Dead ragweed roots generally yielded pure cultures of Rhizoctonia and were not colonized by Corynespora. Koch's postulates were completed in the greenhouse where typical hypocotyl lesions developed in 3–5 days and lateral, "fine feeder roots" were extensively rotted by Corynespora. Soybean yield reduction was related to the density of glyphosate-killed ragweed plants and ranged from 1.5 kg per dead ragweed to 6 kg per dead ragweed in replicated field plots with and without killed ragweed plants. These field observations indicate that glyphosate or metabolites in dying ragweed root exudates modify the soil environment to predispose adjacent glyphosate-resistant soybean roots to severe Corynespora root rot even at temperatures above 20°C.

http://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO.2005.95.6.S1

Kremer, R.; Means, N.; Kim, S. 2005. Glyphosate affects soybean root exudation and rhizosphere microorganisms. *Int. J. of Environmental Analytical Chemistry,* 85 (15): 1165-1174.

Glyphosate is a nonselective, broad-spectrum herbicide that kills plants by inhibiting the enzyme 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), which is necessary for synthesis of aromatic amino acids. A secondary mode of action involves infection of roots by soilborne microorganisms due to decreased production of plant protection compounds known as phytoalexins. Varieties of several crops, including glyphosate-resistant (GR) or Roundup Ready® soybean, are genetically modified to resist the herbicidal effects of glyphosate and provide farmers with an effective weed management tool. After glyphosate is applied to GR soybean, glyphosate that is not bound to glyphosate-resistant EPSPS is translocated throughout the plant and accumulates primarily in meristematic tissues. We previously reported that fungal colonization of GR soybean roots increased significantly after application of glyphosate but not after conventional postemergence herbicides. Because glyphosate may be released into soil from GR roots, we characterized the response of rhizosphere fungi and bacteria to root exudates from GR and non-GR cultivars treated with and without glyphosate at field application rates. Using an immunoassay technique, the flux of glyphosate detected in exudates of hydroponically-grown GR soybean was > 1000 ng plant-1 over the 16-d post-glyphosate application period. Glyphosate also increased carbohydrate and amino acid contents in root exudates in both soybean cultivars. However, GR soybean released higher carbohydrate and amino acid contents in root exudates than non-GR soybean without glyphosate treatment. In vitro bioassays showed that glyphosate in the exudates stimulated growth of selected rhizosphere fungi, possibly by providing a selective C and N source combined with the high levels of soluble carbohydrates and amino acids associated with glyphosate treatment of the soybean plants. Increased fungal populations that develop under glyphosate treatment of GR soybean may adversely affect plant growth and biological processes in the soil and rhizosphere.

Artigo completo disponível em http://afrsweb.usda.gov/sp2UserFiles/Place/50701000/cswq-0210-170287.pdf

Neumann, G.; Kohls, S.; Landsberg, E.; Souza, K.; Yamada, T.; Romheld, V. 2006. Relevance of glyphosate transfer to non-target plants via the rhizosphere. *Journal of Plant Diseases and Protection,* 20: 963–969.

There is a common understanding that the widely used herbicide glyphosate is easily degraded and adsorbed in soils and thus, harmless for use in agriculture. We can demonstrate, however, that this conclusion is wrong and dangerous for farmers because in former risk assessments the behaviour of glyphosate in the rhizosphere was not properly considered. In nutrient solution, rhizobox and pot experiments we can show that foliar applied glyphosate to target plants is released into the rhizosphere after a fast translocation from shoots to roots. In the rhizosphere glyphosate can obviously be stabilized long enough to achieve negative effects on non-target plants. Such a negative side effect is for example inhibited acquisition of micronutrients such as Mn, but also Zn, Fe and B, which are involved in plant own disease resistance mechanisms. From this glyphosate transfer from target to non-target plants (e.g. from weed to trees in orchards) we predict an increase in disease problems, particularly on soils with low micronutrient availability as already reported in the USA. In view of plant and soil health, we urgently call for a re-assessment of glyphosate as herbicide.

Artigo completo disponível em http://www.jpdp-online.com/artikel.dll/02-Roemheld_MTAyNzEw.PDF?UID=DD983A5A02BBFE34816E2BCA8AF8D679AD1AD836C9F3A9B376

Zablotowicz, R.; Reddy, K. 2007. Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. *Crop Prot,* 26:370-6.

Transgenic glyphosate-resistant (GR) soybean [*Glycine max* (L.) Merr.] expressing a glyphosate-insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme has provided new opportunities for weed control in soybean production. However, glyphosate is toxic to the soybean nitrogen-fixing symbiont, *Bradyrhizobium japonicum,* as its EPSPS enzyme is sensitive to glyphosate. The effects of glyphosate on symbiotic parameters, nitrogen accumulation, and yield in GR soybean under weed-free conditions were determined in a 3-yr field study during 2002–2004. Four glyphosate (0.84, 1.68, 2.52+2.52, and 0.84+0.84 kg ae/ha) treatments applied at 4 and 6 weeks after planting (WAP) soybean were compared to a no glyphosate, hand weeded (weed-free) control. In 2002 and 2003, soybean plants were harvested at 5, 6, 7, and 8 WAP, and roots assessed for nitrogenase activity (acetylene reduction assay, ARA), root respiration, nodulation, and root biomass. Soybean seed yield, leaf and seed nitrogen content were determined in all three years. No consistent effect of glyphosate was observed on either ARA or root respiration. In 2002, both ARA and respiration were about a third of that in 2003, attributed to early-season drought in 2002. All glyphosate treatments reduced foliar nitrogen content (26–42%) in 2002. In 2003 and 2004, three and two glyphosate treatments, respectively, reduced foliar nitrogen content (8–13%), with the greatest reduction when glyphosate was applied at the highest rate. Soybean yield was reduced by 11% with two applications of 2.52 kg ae/ha glyphosate compared to hand weeded control in 2002, but seed yield was not affected in 2003 and 2004. Total seed nitrogen harvested in 2002 and 2003 was reduced by 32% and 17%, respectively, when two applications of 2.52 kg ae/ha glyphosate were applied compared to hand weeded soybean. These studies indicate that nitrogen fixation and/or assimilation in GR soybean was only slightly affected at label use rate, but was consistently reduced at above label use rates of glyphosate and the greatest reductions occurred with soil moisture stress following glyphosate application.

http://www.sciencedirect.com/science/article/pii/S0261219406002584

Bott, S.; Tesfamariam, T.; Candan, H.; Cakmak, I.; Römheld, V.; Neumann, G. 2008. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). *Plant Soil,* 312:185–194.

This investigation demonstrated potential detrimental side effects of glyphosate on plant growth and micronutrient (Mn, Zn) status of a glyphosate-resistant (GR) soybean variety (Glycine max cv. Valiosa), which were found to be highly dependent on the selected growth conditions. In hydroponic experiments with sufficient Mn supply [0.5 µM], the GR cv. Valiosa produced similar plant biomass, root length and number of lateral roots in the control treatment without glyphosate as compared to its non-GR parental line cv. Conquista. However, this was associated with 50% lower Mn shoot concentrations in cv. Conquista, suggesting a higher Mn demand of the transgenic cv. Valiosa under the selected growth conditions. Glyphosate application significantly inhibited root biomass production, root elongation, and lateral root formation of the GR line, associated with a 50% reduction of Mn shoot concentrations. Interestingly, no comparable effects were detectable at low Mn supply [0.1 µM]. This may indicate Mn-dependent differences in the intracellular transformation of glyphosate to the toxic metabolite aminomethylphosphonic acid (AMPA) in the two isolines. In soil culture experiments conducted on a calcareous loess sub-soil of a Luvisol (pH 7.6) and a highly weathered Arenosol (pH 4.5), shoot biomass production and Zn leaf concentrations of the GR-variety were affected by glyphosate applications on the Arenosol but not on the calcareous Loess sub-soil. Analysis of micronutrient levels in high and low molecular weight (LMW) fractions (80% ethanol extracts) of young leaves revealed no indications for internal immobilization of micronutrients (Mn, Zn, Fe) by excessive complexation with glyphosate in the LMW phase.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/GlyphosateBott.pdf

Johal, G.; Huber, D. 2009. Glyphosate effects on diseases of plants. *Europ. J. Agronomy,* 31, 144-152.

Glyphosate, N-(phosphonomethyl) glycine, is the most extensively used herbicide in the history of agriculture. Weed management programs in glyphosate resistant (GR) field crops have provided highly effective weed control, simplified management decisions, and given cleaner harvested products. However, this relatively simple, broad-spectrum, systemic herbicide can have extensive unintended effects on nutrient efficiency and disease severity, thereby threatening its agricultural sustainability. A significant increase in disease severity associated with the wide spread application of the glyphosate herbicide can be the result of direct glyphosate-induced weakening of plant defenses and increased pathogen population and virulence. Indirect effects of glyphosate on disease predisposition result from immobilization of specific micronutrients involved in disease resistance, reduced growth and vigor of the plant from accumulation of glyphosate in meristematic root, shoot, and reproductive tissues, altered physiological efficiency, or modification of the soil microflora affecting the availability of nutrients involved in physiological disease resistance. Strategies to ameliorate the predisposing effects of glyphosate on disease include judicious selection of herbicide application rates, micronutrient amendment, glyphosate detoxification in meristematic tissues and soil, changes in cultural practices to enhance micronutrient availability for plant uptake, and biological amendment with glyphosate-resistant microbes for nitrogen fixation and nutrient availability. Given that recommended doses of glyphosate are often many times higher than needed to control weeds, we believe the most prudent method to reduce the detrimental effects of glyphosate on GR crops will be to use this herbicide in as small a dose as practically needed. Such a frugal approach will not only curtail disease predisposition of GR crops, but will also benefit the grower and the environment.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/webfm/plataforma/GlyphosateEffectsDiseases.pdf

Fernandez, M.; Zentner, R.; Basnyat, P.; Gehl, D.; Selles, F.; Huber, D. 2009. Glyphosate associations with cereal diseases caused by Fusarium spp. in the Canadian prairies. *European Journal of Agronomy*, 31: 133–143.

Fusarium pathogens cause important diseases, such as root/crown rot and Fusarium head blight (FHB), in cereal crops. These diseases can be caused by similar *Fusarium* spp. Common root rot (CRR) is widespread in the western Canadian Prairies, whereas FHB has potential of becoming an important disease in this region. There are no commercially available cereal cultivars with good resistance to these diseases. It is therefore important to identify agronomic practices that could affect levels of *Fusarium* pathogens in cereals. This review deals primarily with the effects of tillage systems and glyphosate use on the development of FHB and CRR in wheat and barley in eastern Saskatchewan. Although the FHB study in 1999–2002 indicated that environment was the most important factor determining FHB development, previous glyphosate use and tillage practice were among the production factors with the greatest association with FHB. Overall, disease was highest in crops under minimum-till management. Previous glyphosate use was consistently associated with higher FHB levels caused by the most important FHB pathogens, *Fusarium avenaceum* and *Fusarium graminearum. Cochliobolus sativus*, the most common CRR pathogen, was negatively associated with previous glyphosate use, while *F. avenaceum,F. graminearum*, and other fungi were positively associated, suggesting that glyphosate might cause changes in fungal communities. The occurrence and isolation of *F. avenaceum* from cereal residues were greater under reduced-till than conventional-till while *C. sativus* was most common under conventional-till, and *F. graminearum* was lowest under zero-till. Previous glyphosate applications were again correlated positively with *F. avenaceum* and negatively with *C. sativus*. These observations agreed with results from the FHB and CRR studies. These are the first studies that established a relationship between previous glyphosate use and increased *Fusarium* infection of spikes and subcrown internodes of wheat and barley, or *Fusarium* colonization of crop residues. However, because of the close association between noncereal crops, reduced tillage and glyphosate use, it was not possible to completely separate the effects of these factors on *Fusarium* infections. Determining the relative contribution of these popular production trends to the development of diseases caused by *Fusarium* spp. are essential for devising appropriate agronomic recommendations to prevent their further spread in western Canada, and to reduce the impact that these diseases are having in areas where they are already established. The consistent association between previous glyphosate use and *Fusarium* infections also warrants further research to elucidate the nature of this association and the underlying mechanisms determining these effects.

http://www.researchgate.net/publication/222122783_Glyphosate_associations_with_cereal_diseases_caused_by_Fusarium_spp._in_the_Canadian_Prairies

Kremer, R.; Means, N. 2009. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. *European Journal of Agronomy,* 31 (3): 153-161.

Current crop production relies heavily on transgenic, glyphosate-resistant (GR) cultivars. Widespread cultivation of transgenic crops has received considerable attention. Impacts of glyphosate on rhizosphere microorganisms and activities are reviewed based on published and new data from long-term field projects documenting effects of glyphosate applied to GR soybean and maize. Field studies conducted in Missouri, U.S.A. during 1997–2007 assessed effects of glyphosate applied to GR soybean and maize on root colonization and soil populations of *Fusarium* and selected rhizosphere bacteria. Frequency of root-colonizing *Fusarium* increased significantly after glyphosate application during growing seasons in each year at all sites. Roots of GR soybean and maize treated with glyphosate were heavily colonized by *Fusarium* compared to non-GR or GR cultivars not treated with glyphosate. Microbial groups and functions affected by glyphosate included Mn transformation and plant availability; phytopathogen–antagonistic bacterial interactions; and reduction in nodulation. Root-exuded glyphosate may serve as a nutrient source for fungi and stimulate propagule germination. The specific microbial indicator groups and processes were sensitive to impacts of GR crops and are part of an evolving framework in developing polyphasic microbial analyses for complete assessment of GR technology that is more reliable than single techniques or general microbial assays.

http://www.sciencedirect.com/science/article/pii/S1161030109000641

Zobiole, L.; Oliveira, R.; Visentainer, J.; Kremer, R.; Bellaloui, N.; Yamada, T. 2010. Glyphosate affects seed composition in glyphosate-resistant soybean. *J. Agric. Food Chem.,* 58 (7), 4517-4522.

The cultivation of glyphosate-resistant (GR) soybeans has continuously increased worldwide in recent years mainly due to the importance of glyphosate in current weed management systems. However, not much has been done to understand eventual effects of glyphosate application on GR soybean physiology, especially those related to seed composition with potential effects on human health. Two experiments were conducted to evaluate the effects of glyphosate application on GR soybeans compared with its near-isogenic non-GR parental lines. Results of the first experiment showed that glyphosate application resulted in significant decreases in shoot nutrient concentrations, photosynthetic parameters, and biomass production. Similar trends were observed for the second experiment, although glyphosate application significantly altered seed nutrient concentrations and polyunsaturated fatty acid percentages. Glyphosate resulted in significant decreases in polyunsaturated linoleic acid (18:2n-6) (2.3% decrease) and linolenic acid (18:3n-3) (9.6% decrease) and a significant increase in monounsaturated fatty acids 17:1n-7 (30.3% increase) and 18:1n-7 (25% increase). The combined observations of decreased photosynthetic parameters and low nutrient availability in glyphosate-treated plants may explain potential adverse effects of glyphosate in GR soybeans.

http://www.ncbi.nlm.nih.gov/pubmed/20307082

Zobiole, L.; Bonini, E.; Oliveira, R.; Kremer, R. ; Ferrarese, O. 2010. Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean. *Acta Physiologiae Plantarum*, 32, 831–837.

Farmers report that some glyphosate-resistant soybean varieties are visually injured by glyphosate. Glyphosate is the main herbicide that directly affects the synthesis of secondary compounds. In this work, we evaluated the effect of increasing rates of glyphosate on lignin and amino acid content, photosynthetic parameters and dry biomass in the early maturity group cultivar BRS 242 GR soybean. Plants were grown in half-strength complete nutrient solution and subjected to various rates of glyphosate either as a single or in sequential applications. All parameters evaluated were affected by increasing glyphosate rates. The effects were more pronounced as glyphosate rates increased, and were more intense with a single total application than sequential applications at lower rates.

http://link.springer.com/article/10.1007%2Fs11738-010-0467-0

Zobiole, L.; Oliveira, R.; Kremer, R.; Constantin, J.; Yamada, T.; Castro, C.; Oliveira, F.; Oliveira, A.; 2010. Effect of glyphosate on symbiotic N2 fixation and nickel concentration in glyphosate-resistant soybeans. *Applied Soil Ecology*, 44: 176-180.

Decreased biological nitrogen fixation in glyphosate-resistant (GR) soybeans has been attributed directly to toxicity of glyphosate or its metabolites, to N2-fixing microorganisms. As a strong metal chelator, glyphosate could influence symbiotic N2 fixation by lowering the concentration of nickel (Ni) that is essential for the symbiotic microorganisms. Evaluation of different cultivars grown on different soil types at the State University of Maringa´ , PR, Brazil during the summer 2008 revealed, significant decreases in photosynthetic parameters (chlorophyll, photosynthetic rate, transpiration and stomatal conductance) and nickel content with glyphosate use (single or sequential application). This work demonstrated that glyphosate can influence the symbiotic N2 fixation by lowering nickel content available to the symbiotic microorganisms.

Artigo completo dispnível em http://ainfo.cnptia.embrapa.br/digital/bitstream/item/94962/1/Effect-of-glyphosate-on-symbiotic-N2-fixation-and-nickel-concentration-in-glyphosate-resistant-soybeans.pdf

Zobiole, L.; Kremer, R.; Oliveira, R.; Constantin, J. 2011. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. *Journal of Applied Microbiology*, 110: 118-127.

Aims: Glyphosate-resistant (GR) soybean production increases each year because of the efficacy of glyphosate for weed management. A new or ‘second’ generation of GR soybean (GR2) is now commercially available for farmers that is being promoted as higher yielding relative to the previous, ‘first generation’ (GR1) cultivars. Recent reports show that glyphosate affects the biology and ecology of rhizosphere micro-organisms in GR soybean that affect yield. The objective of this research was to evaluate the microbiological interactions in the rhizospheres of GR2 and GR1 soybean and the performance of the cultivars with different rates of glyphosate applied at different growth stages.
Methods and Results: A greenhouse study was conducted using GR1 and GR2 soybean cultivars grown in a silt loam soil. Glyphosate was applied at V2, V4 and V6 growth stages at three rates. Plants harvested at R1 growth stage had high root colonization by Fusarium spp.; reduced rhizosphere fluorescent pseudomonads, Mn-reducing bacteria, and indoleacetic acid–producing rhizobacteria; and reduced shoot and root biomass.
Conclusions: Glyphosate applied to GR soybean, regardless of cultivar, negatively impacts the complex interactions of microbial groups, biochemical activity and root growth that can have subsequent detrimental effects on plant growth and productivity.
Significance and Impact of the Study: The information presented here will be crucial in developing strategies to overcome the potential detrimental effects of glyphosate in GR cropping systems.

Artigo completo disponível em http://www.dag.uem.br/napd/up/Public-NAPD_cd97d9606b1001b39a19da9d6214790fqqabj.pdf

3.2.2 Danos agronômicos em lavouras adjacentes e/ou subsequentes

Estudos mostram que, em função da deriva, na forma de microgotas aerotransportadas, e/ou devido a seu transporte em águas superficiais ou ainda por meio de sua acumulação no solo (afetando rizosfera e micorrizas), o glifosato, o 2,4-D, o dicamba e outros herbicidas podem causar danos agronômicos relevantes tanto para o cultivo onde estão sendo aplicados como também em lavouras subsequentes ou adjacentes.

Eker, S.; Ozturk, L.; Yazici, A.; Erenoglu, B.; Romheld, V.; Cakmak, I. 2006. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus L.*) plants. *J. Agric. Food Chem.*, 54 (26). pp 10019-10025.

Evidence clearly shows that cationic micronutrients in spray solutions reduce the herbicidal effectiveness of glyphosate for weed control due to the formation of metal-glyphosate complexes. The formation of these glyphosate-metal complexes in plant tissue may also impair micronutrient nutrition of nontarget plants when exposed to glyphosate drift or glyphosate residues in soil. In the present study, the effects of simulated glyphosate drift on plant growth and uptake, translocation, and accumulation (tissue concentration) of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were investigated in sunflower (Helianthus annuus L.) plants grown in nutrient solution under controlled environmental conditions. Glyphosate was sprayed on plant shoots at different rates between 1.25 and 6.0% of the recommended dosage (i.e., 0.39 and 1.89 mM glyphosate isopropylamine salt). Glyphosate applications significantly decreased root and shoot dry matter production and chlorophyll concentrations of young leaves and shoot tips. The basal parts of the youngest leaves and shoot tips were severely chlorotic. These effects became apparent within 48 h after the glyphosate spray. Glyphosate also caused substantial decreases in leaf concentration of Fe and Mn while the concentration of Zn and Cu was less affected. In short-term uptake experiments with radiolabeled Fe (59Fe), Mn (54Mn), and Zn (65Zn), root uptake of 59Fe and 54Mn was significantly reduced in 12 and 24 h after application of 6% of the recommended dosage of glyphosate, respectively. Glyphosate resulted in almost complete inhibition of root-to-shoot translocation of 59Fe within 12 h and 54Mn within 24 h after application. These results suggest that glyphosate residues or drift may result in severe impairments in Fe and Mn nutrition of nontarget plants, possibly due to the formation of poorly soluble glyphosate-metal complexes in plant tissues and/or rhizosphere interactions.

http://www.ncbi.nlm.nih.gov/pubmed/17177536

Cakmak I., Yazici A., Tutus Y., Ozturk L. (2009) Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *Eur J Agron,* 31(3):114-9.

Greenhouse experiments were conducted to study the effects of glyphosate drift on plant growth and concentrations of mineral nutrients in leaves and seeds of non-glyphosate resistant soybean plants (*Glycine max*, L.). Glyphosate was sprayed on plant shoots at increasing rates between 0.06 and 1.2% of the recommended application rate for weed control. In an experiment with 3-week-old plants, increasing application of glyphosate on shoots significantly reduced chlorophyll concentration of the young leaves and shoots dry weight, particularly the young parts of plants. Concentration of shikimate due to increasing glyphosate rates was nearly 2-fold for older leaves and 16-fold for younger leaves compared to the control plants without glyphosate spray. Among the mineral nutrients analyzed, the leaf concentrations of potassium (K), phosphorus (P), copper (Cu) and zinc (Zn) were not affected, or even increased significantly in case of P and Cu in young leaves by glyphosate, while the concentrations of calcium (Ca), manganese (Mn) and magnesium (Mg) were reduced, particularly in young leaves. In the case of Fe, leaf concentrations showed a tendency to be reduced by glyphosate. In the second experiment harvested at the grain maturation, glyphosate application did not reduce the seed concentrations of nitrogen (N), K, P, Zn and Cu. Even, at the highest application rate of glyphosate, seed concentrations of N, K, Zn and Cu were increased by glyphosate. By contrast, the seed concentrations of Ca, Mg, Fe and Mn were significantly reduced by glyphosate. These results suggested that glyphosate may interfere with uptake and retranslocation of Ca, Mg, Fe and Mn, most probably by binding and thus immobilizing them. The decreases in seed concentration of Fe, Mn, Ca and Mg by glyphosate are very specific, and may affect seed quality.

http://www.sciencedirect.com/science/article/pii/S1161030109000665

Egan, J.; Barlow, K.; Mortensen, D. 2014. A Meta-Analysis on the Effects of 2,4-D and Dicamba Drift on Soybean and Cotton. *Weed Science*, 62(1):193-206. 2014.

Commercial introduction of cultivars of soybean and cotton genetically modified with resistance to the synthetic auxin herbicides dicamba and 2,4-D will allow these compounds to be used with greater flexibility but may expose susceptible soybean and cotton cultivars to nontarget herbicide drift. From past experience, it is well known that soybean and cotton are both highly sensitive to low-dose exposures of dicamba and 2,4-D. In this study, a meta-analysis approach was used to synthesize data from over seven decades of simulated drift experiments in which investigators treated soybean and cotton with low doses of dicamba and 2,4-D and measured the resulting yields. These data were used to produce global dose–response curves for each crop and herbicide, with crop yield plotted against herbicide dose. The meta-analysis showed that soybean is more susceptible to dicamba in the flowering stage and relatively tolerant to 2,4-D at all growth stages. Conversely, cotton is tolerant to dicamba but extremely sensitive to 2,4-D, especially in the vegetative and preflowering squaring stages. Both crops are highly variable in their responses to synthetic auxin herbicide exposure, with soil moisture and air temperature at the time of exposure identified as key factors. Visual injury symptoms, especially during vegetative stages, are not predictive of final yield loss. Global dose–response curves generated by this meta-analysis can inform guidelines for herbicide applications and provide producers and agricultural professionals with a benchmark of the mean and range of crop yield loss that can be expected from drift or other nontarget exposures to 2,4-D or dicamba.

http://www.bioone.org/doi/abs/10.1614/WS-D-13-00025.1

Carvalho, F.; Souza, B.; França, A.; Ferreira, E.; Franco, M.; Kasuya, M.; Ferreira, F. 2014. Glyphosate drift affects arbuscular mycorrhizal association in coffee. *Planta daninha*, vol.32 nº 4, Viçosa.

Mycorrhizal association promotes better survival and nutrition of colonized seedling on field, and consequently, increasing of productivity. However, the weed management can interfere on this association, due to incorrect use of glyphosate. This work has assessed the effects of glyphosate drift on the growth and nutrition of arabica coffee plants (Catuaí Vermelho - IAC 99) colonized with arbuscular mycorrhizal fungi (AMF). The experiment was conducted in 2 x 5 factorial scheme, and included inoculated and non-inoculated plants, and five glyphosate subdoses (0.0, 57.6, 115.2, 230.4, and 460.8 g ha^{-1} of glyphosate), in randomized blocks with five replication. The inoculation was carried during the greenhouse phase of seedlings production with a mixture of *Rhizophagus clarus* and *Gigaspora margarita*, and after to transplanting, when the plants had seven pairs of leaves, glyphosate subdoses were applied. The product caused intoxication in up to 60% of non-inoculated and 45% on inoculated plants, when the highest dose of 460.8 g a.e. ha^{-1} was applied. A negative effect was noted on the growth and phosphorus content of coffee plants, this effect increased depending on glyphosate subdose, but regardless of inoculation. Glyphosate drift reduces the growth and nutrition of plants colonized by species of AMF and native fungi, negatively affecting root colonization of plants treated.

Artigo completo disponível em http://www.scielo.br/pdf/pd/v32n4/13.pdf

3.3 Disseminação transgênica no agrossistema: quando a tecnologia é uma praga [37]

Em alguns casos, o transgene inserido na PGM, quando introgredido em espécies geneticamente aparentadas e consideradas pragas (ou daninhas[38] ou *weedy* em inglês), pode lhe conferir uma vantagem adaptativa, gerando graves problemas de manejo. De fato, as ervas daninhas supostamente controladas pelo herbicida ao qual a planta TH é tolerante sobrevivem ao tratamento, inviabilizando a tecnologia. Adicionalmente, em sucessão de lavouras de milho, soja e algodão RR, sementes caídas em uma colheita podem germinar em paralelo ao novo plantio, criando dificuldades de controle na medida em que também serão resistentes aos glifosatos ou aos herbicidas envolvidos com aquela e com outras tecnologias (caso do glufosinato, nas lavouras Liberty Link).

Os artigos listados a seguir ilustram os mecanismos responsáveis por problemas agronômicos decorrentes da transferência do transgene de resistência, desde as lavouras pretendidas até algumas espécies ruderais que se pretenderia controlar.

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[37] Cabe salientar que essa parte divide uma temática comum com o item 3.2 da Parte 3 que diz respeito a escape de transgenes em espécies geneticamente aparentadas e selvagens. Por vezes, a distinção entre essas populações selvagens/naturais e aquelas ruderais/adventícias é ambígua, em especial no caso de populações ferais que são justamente populações de plantas domesticadas em processo de renaturalização.

[38] A expressão ruderal empregada ao longo desta publicação é no sentido proposto por Schneider (2007) (Schneider, A. A. A flora naturalizada no estado do Rio Grande do Sul, Brasil: herbáceas subespontâneas**.** *Biociências*, Porto Alegre, v. 15, nº 2, p. 257-268, jul. 2007) e diz respeito às espécies vegetais que se desenvolvem sem cultivo e sem cuidado humano, englobando tanto as espécies nativas (autóctones) quanto as naturalizadas. Ao contrário do termo "daninho", ruderal não tem juízo de valor e recusa a premissa falsa de que qualquer planta que não seja a cultura objeto seria prejudicial, o que não corresponde à verdade dos sistemas naturais que têm a diversidade como elemento inerente essencial à homeostase.

Hall, L.; Topinka, K.; Huffman, J.; Davis, L.; Good, A. 2000. Pollen flow between herbicide-resistant Brassica napus is the cause of multiple-resistant B-napus volunteers. *Weed Science,* 48: 688-694.

A field in which *Brassica napus* volunteers were not controlled by several applications of glyphosate was investigated in 1998. This field had been planted with glufosinateresistant and imidazolinone-resistant *B. napus* in 1997 and was adjacent to a field that had grown glyphosate-resistant *B. napus.* Mature volunteer *B. napus* were collected on a 50- by 100-m grid in the field. Progeny from 34 volunteers were sprayed with glyphosate at 440 g ae ha_1, and the survivors were sprayed with either glufosinate or imazethapyr at 400 or 50 g ai ha_1, respectively. Where seed numbers permitted (14 volunteers), seedlings were also sprayed sequentially with glyphosate, glufosinate, and imazethapyr, at 440 g ae ha_1, 400 g ai ha_1, and 50 g ai ha_1, respectively. In total, 15 volunteers had progeny that were between 66 and 82% resistant to glyphosate, consistent with the predicted 3:1 resistant : susceptible ratio. Volunteer *B. napus* plants with glyphosate-resistant seedlings were most common close to the putative pollen source; however, a plant with glyphosate-resistant progeny was collected 500 m from the adjacent field edge. Seedlings from all nine volunteers collected from the glufosinate-resistant area showed multiple resistance to glyphosate and glufosinate, whereas seedlings from 10 of 20 volunteers collected from the imidazolinone-resistant area showed resistance to imazethapyr and glyphosate. DNAextraction and restriction fragment length polymorphism (RFLP) analysis of seedlings confirmed that mature *B. napus* volunteers were hybrids resulting from pollen transfer rather than inadvertent seed movement between fields. Two seedlings from the 924 screened were resistant to all three herbicides. Progeny from these selfpollinated individuals were resistant to glyphosate and glufosinate at the predicted 3:1 resistant : susceptible ratio and resistant to imazethapyr at the predicted 15:1 resistant : susceptible ratio. Sequential crossing of three herbicide-resistant varieties is the most likely explanation for the observed multiple herbicide resistance. Integrated management techniques, including suitable crop and herbicide rotations, herbicide mixtures, and nonchemical controls should be used to reduce the incidence and negative effect of *B. napus* volunteers with multiple herbicide resistance.

http://www.jstor.org/discover/10.2307/4046338?sid=21105684013393&uid=2&uid=4&uid=3737664

Arnaud, J.; Viard, F.; Delescluse, M.; Cuguen, J. 2003. Evidence for gene flow via seed dispersal from crop to wild relatives in *Beta vulgaris* (Chenopodiaceae): consequences for the release of genetically modified crop species with weedy lineages. *Proc. R. Soc. Lond. B*, 270, 1565–1571.

Gene flow and introgression from cultivated to wild plant populations have important evolutionary and ecological consequences and require detailed investigations for risk assessments of transgene escape into natural ecosystems. Sugar beets (Beta vulgaris ssp. vulgaris) are of particular concern because: (i) they are cross-compatible with their wild relatives (the sea beet, B. vulgaris ssp. maritima); (ii) crop-to-wild gene flow is likely to occur via weedy lineages resulting from hybridization events and locally infesting fields. Using a chloroplastic marker and a set of nuclear microsatellite loci, the occurrence of crop-to-wild gene flow was investigated in the French sugar beet production area within a 'contact-zone' in between coastal wild populations and sugar beet fields. The results did not reveal large pollen dispersal from weed to wild beets. However, several pieces of evidence clearly show an escape of weedy lineages from fields via seed flow. Since most studies involving the assessment of transgene escape from crops to wild outcrossing relatives generally focused only on pollen dispersal, this last result was unexpected: it points out the key role of a long-lived seed bank and highlights support for transgene escape via man-mediated long-distance dispersal events.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1691408/pdf/12908976.pdf

Gealy, D.; Mitten, D.; Rutger, J. 2003. Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*)-Implications for weed management. *Weed Technology,* vol. 17: 627-645.

Red rice has long been a troublesome, conspecific weed of cultivated rice. Rice varieties carrying certain herbicide-resistant traits acquired through genetic modification (herbicide-resistant varieties) now offer new options for red rice control. In concert with this innovation is the risk of gene flow, which can result in the transfer of that specific herbicide resistance to red rice and thus render this weed control measure ineffective. Gene flow in concept is simple, however, the parameters that determine the establishment of a new trait in a weed population are complex. Cross-pollination to make hybrid seed and the subsequent fate of those hybrid families in the general weed population are some of the biological factors that influence gene flow between red rice and cultivated rice. Natural outcrossing among rice plants is generally low. Most of the pollen dispersal studies published to date indicated that rice 3 rice outcrossing rates were less than 1.0%. Numerous reports summarized in this study suggest that outcrossing rates between rice and red rice can be highly variable but usually are similar to or lower than this level. However, once hybrids form, they may introgress into a red rice population within only a few generations. If hybrid seed families are to persist and establish herbicide-resistant red rice populations, they must successfully compete in the crop–weed complex. The ability to survive a herbicide applied to a herbicide-resistant rice variety would be a strong selective advantage for these hybrid families. Thus, the well-established principles of weed resistance management appear to be relevant for herbicide-resistant crop systems and should be used in combination with practices to minimize coincident flowering to mitigate the potential impact of gene flow from herbicide-resistant rice into red rice. For the rice–red rice crop–weed complex, there are both biological factors and agricultural practices that can work together to preserve these new weed control options.

Artigo completo disponível em http://naldc.nal.usda.gov/naldc/download.xhtml?id=54993&content=PDF

Messeguer, J.; Marfa, V.; Catala, M.; Guiderdoni, E.; Melé, E. 2004. A field study of pollen-mediated gene flow from Mediterranean GM rice to conventional rice and the red rice weed. *Molecular Breeding*, 13 (1), 103- 112.

The objective of this study was to assess the frequency of pollen-mediated gene flow from a transgenic rice line, harbouring the *gusA* and the *bar* genes encoding respectively, _-glucuronidase and phosphinothricin acetyl transferase as markers, to the red rice weed and conventional rice in the Spanish *japonica* cultivar Senia. A circular field trial design was set up to investigate the influence of the wind on the frequency of pollination of red rice and conventional rice recipient plants with the transgenic pollen. Frequencies of gene flow based on detection of herbicide resistant, GUS positive seedlings among seed progenies of recipient plants averaged over all wind directions were 0.036 _ 0.006% and 0.086 _ 0.007 for red rice and conventional rice, respectively. However, for both red rice and conventional rice, a clear asymmetric distribution was observed with pollination frequency favoured in plants placed under the local prevailing winds. Southern analyses confirmed the hemizygous status and the origin of the transgenes in progenies of surviving, GUS positive plants. Gene flow detected in conventional rice planted at 1, 2, 5 and 10 m distance revealed a clear decrease with increasing distance which was less dramatic under the prevailing wind direction. Consequences of these findings for containment of gene flow from transgenic rice crops to the red rice weed are discussed. The precise determination of the local wind conditions at flowering time and pollination day time appear to be of primary importance for setting up suitable isolation distances.

http://link.springer.com/article/10.1023%2FB%3AMOLB.0000012285.39859.9d

Légère, A. 2005. Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus* L.) as a case study. *Pest Manag. Sci.,* 61 (3): 292-300.

Data from the literature and recent experiments with herbicide-resistant (HR) canola (Brassica napus L) repeatedly confirm that genes and transgenes will flow and hybrids will form if certain conditions are met. These include sympatry with a compatible relative (weedy, wild or crop), synchrony of flowering, successful fertilization and viable offspring. The chance of these events occurring is real; however, it is generally low and varies with species and circumstances. Plants of the same species (non-transgenic or with a different HR transgene) in neighbouring fields may inherit the new HR gene, potentially generating plants with single and multiple HR. For canola, seed losses at harvest and secondary dormancy ensures the persistence over time of the HR trait(s) in the seed bank, and the potential presence of crop volunteers in subsequent crops. Although canola has many wild/weedy relatives, the risk of gene flow is quite low for most of these species, except with Brassica rapa L. Introgression of genes and transgenes in B rapa populations occurs with apparently little or no fitness costs. Consequences of HR canola gene flow for the agro-ecosystem include contamination of seed lots, potentially more complex and costly control strategy, and limitations in cropping system design. Consequences for non-agricultural habitats may be minor but appear largely undocumented.

http://www.ncbi.nlm.nih.gov/pubmed/15593291

Zelaya, I.; Owen, M.; VanGessel, M. 2007. Transfer of glyphosate-resistance: Evidence of hybridisation in *Conyza* (Asteraceae). *American Journal of Botany*, 94: 660-673.

Transfer of herbicide resistance genes between crops and weeds is relatively well documented; however, far less information exists for weed-to-weed interactions. The hybridization between the weedy diploids Conyza canadensis (2n ¼ 18) and C. ramosissima (2n ¼ 18) was investigated by monitoring transmission of the allele conferring resistance to N-phosphonomethyl glycine (glyphosate). In a multivariate quantitative trait analysis, we described the phylogenic relationship of the plants, whereas we tested seed viability to assess potential postzygotic reproductive barriers (PZRB) thus affecting the potential establishment of hybrid populations in the wild. When inflorescences were allowed to interact freely, approximately 3% of C. ramosissima or C. canadensis ova were fertilized by pollen of the opposing species and produced viable seeds; > 95% of the ova were fertilized under no-pollen competition conditions (emasculation). The interspecific Conyza hybrid (F^{H}_{1}) demonstrated an intermediate phenotype between the parents but superior resistance to glyphosate compared to the resistant C. canadensis parent. Inheritance of glyphosate resistance in the selfed F^{H}_{1} (F^{H}_{2}) followed the partially dominant nuclear, single-gene model; F^{H}_{1} backcrosses confirmed successful introgression of the resistance allele to either parent. Negligible PZRB were observed in the hybrid progenies, confirming fertility of the C. canadensis 3 C. ramosissima nothotaxa. The implications of introgressive hybridization for herbicide resistance management and taxonomy of Conyza are discussed.

Artigo completo disponível em http://www.amjbot.org/content/94/4/660.long

Song, X.; Liu, L.; Wang, Z.; Qiang, S. 2009. Potential gene flow from transgenic rice (*Oryza sativa L.*) to different weedy rice (*Oryza sativa f. spontanea*) accessions based on reproductive compatibility. *Pest Management Science, v*ol 65 (8): 862-9.

Background: The possibility of gene flow from transgenic crops to wild relatives may be affected by reproductive capacity between them. The potential gene flow from two transgenic rice lines containing the bar gene to five accessions of weedy rice (WR1-WR5) was determined through examination of reproductive compatibility under controlled pollination.
Results: The pollen grain germination of two transgenic rice lines on the stigma of all weedy rice, rice pollen tube growth down the style and entry into the weedy rice ovary were similar to self-

pollination in weedy rice. However, delayed double fertilisation and embryo abortion in crosses between WR2 and Y0003 were observed. Seed sets between transgenic rice lines and weedy rice varied from 8 to 76%. Although repeated pollination increased seed set significantly, the rank of the seed set between the weedy rice accessions and rice lines was not changed. The germination rates of F(1) hybrids were similar or greater compared with respective females. All F(1) plants expressed glufosinate resistance in the presence of glufosinate selection pressure.
Conclusions: The frequency of gene flow between different weedy rice accessions and transgenic herbicide-resistant rice may differ owing to different reproductive compatibility. This result suggests that, when wild relatives are selected as experimental materials for assessing the gene flow of transgenic rice, it is necessary to address the compatibility between transgenic rice and wild relatives.

http://www.ncbi.nlm.nih.gov/pubmed/19418443

Song, X.; Wang. Z.; Qiang, S. 2011. Agronomic performance of F1, F2 and F3 hybrids between weedy rice and transgenic glufosinate-resistant rice. *Pest Management Science,* 67(8):921-31. doi: 10.1002/ps.2132. Epub 2011 Mar 2.

Background: Studies of hybrid fitness, of which agronomic performance may be an indicator, can help in evaluating the potential for introgression of a transgene from a transgenic crop to wild relatives. The objective of this study was to assess the agronomic performance of reciprocal hybrids between two transgenic glufosinate-resistant rice lines, Y0003 and 99-t, and two weedy rice accessions, WR1 and WR2, in the greenhouse.
Results: F1 hybrids displayed heterosis in height, flag leaf area and number of spikelets per panicle. The agronomic performance of F1 between WR1 and Y0003 was not affected by crossing direction. The tiller and panicle numbers of F1 individuals were higher than their F2 counterparts. However, these traits did not change significantly from the F2 to the F3 generation or in hybrids with weedy rice as maternal or paternal plants. For all hybrids, the in vitro germination rates of fresh pollen were similar and significantly lower than those of their parents, seed sets were similar to or of lower value than those of weedy rice parents and seed shattering characteristics were partially suppressed, but the survival of hybrids over winter in the field was similar to that of weedy rice parents. All F1, F2 and F3 hybrids had similar composite agronomic performance to weedy rice parents.
Conclusion: There was no significant decrease in the composite agronomic performance of any of the hybrids compared with weedy rice. This implies that gene flow from transgenic cultivated rice to weedy rice could occur under natural conditions.

http://www.ncbi.nlm.nih.gov/pubmed/21370396

Em alguns casos, a transferência do transgene em espécies ruderais consideradas pragas (*weedy*, em inglês) pode resultar em vantagem adaptativa dessas plantas, mesmo sem uso do agente de seleção (o herbicida associado a determinada planta TH). Assim, o transgene simplesmente "escapa na natureza", com consequências difíceis de se prever em termos de danos ao meio ambiente no médio e longo prazo.

Warwick, S.; Legere, A.; Simard, M.; James, T. 2008. Do escaped transgenes persist in nature? The case of a herbicide resistance transgene in a weedy Brassica rapa population. *Molecular Ecol., v*ol. 17 (5): 1387-95.

The existence of transgenic hybrids resulting from transgene escape from genetically modified (GM) crops to wild or weedy relatives is well documented but the fate of the transgene over time in recipient wild species populations is still relatively unknown. This is the first report of the persistence and apparent introgression, i.e. stable incorporation of genes from one differentiated gene pool into another, of an herbicide resistance transgene from *Brassica napus* into the gene pool of its weedy relative, *Brassica rapa*, monitored under natural commercial field conditions. Hybridization between glyphosate-resistant [herbicide resistance (HR)] *B. napus* and *B. rapa* was first observed at two Québec sites, Ste Agathe and St Henri, in 2001. *B. rapa* populations at these two locations were monitored in 2002, 2003 and 2005 for the presence of hybrids and transgene persistence. Hybrid numbers decreased over the 3-year period, from 85 out of ~200 plants surveyed in 2002 to only five out of 200 plants in 2005 (St Henri site). Most hybrids had the HR trait, reduced male fertility, intermediate genome structure, and presence of both species-specific amplified fragment length polymorphism markers. Both F1 and backcross hybrid generations were detected. One introgressed individual, i.e. with the HR trait and diploid ploidy level of *B. rapa,* was observed in 2005. The latter had reduced pollen viability but produced ~480 seeds. Forty-eight of the 50 progeny grown from this plant were diploid with high pollen viability and 22 had the transgene (1:1 segregation). These observations confirm the persistence of the HR trait over time. Persistence occurred over a 6-year period, in the absence of herbicide selection pressure (with the exception of possible exposure to glyphosate in 2002), and in spite of the fitness cost associated with hybridization.

http://www.ncbi.nlm.nih.gov/pubmed/17971090

Dlugosch, K.; Whitton, J.; 2008. Can we stop transgenes from taking a walk on the wild side? *Molecular Ecology*, 17(5):1167-9. doi: 10.1111/j.1365-294X.2008.03663.x.

Whether the potential costs associated with broad-scale use of genetically modified organisms (GMOs) outweigh possible benefits is highly contentious, including within the scientific community. Even among those generally in favour of commercialization of GM crops, there is nonetheless broad recognition that transgene escape into the wild should be minimized. But is it possible to achieve containment of engineered genetic elements in the context of large scale agricultural production? In a previous study, Warwick et al. (2003) documented transgene escape via gene flow from herbicide resistant (HR) canola (Brassica napus) into neighbouring weedy B. rapa populations (Fig. 1) in two agricultural fields in Quebec, Canada. In a follow-up study in this issue of Molecular Ecology, Warwick et al. (2008) show that the transgene has persisted and spread within the weedy population in the absence of selection for herbicide resistance. Certainly a trait like herbicide resistance is expected to spread when selected through the use of the herbicide, despite potentially negative epistatic effects on fitness. However, Warwick et al.'s findings suggest that direct selection favouring the transgene is not required for its persistence. So is there any hope of preventing transgene escape into the wild?

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2008.03663.x/pdf

Wang, w.; Xia, H.; Yang, X.; Xu, T.; Si, H.; Cai, X.; Wang, F.; Su, J.; Snow, A.; Lu, B-R. 2013. A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (*Oryza sativa*) without herbicide. *New Phytologist,* doi: 10.1111/nph.12428.

Understanding evolutionary interactions among crops and weeds can facilitate effective weed management. For example, gene flow from crops to their wild or weedy relatives can lead to rapid evolution in recipient populations. In rice (Oryza sativa), transgenic herbicide resistance is expected to spread to conspecific weedy rice (Oryza sativa f. spontanea) via hybridization. Here, we studied fitness effects of transgenic over-expression of a native 5-enolpyruvoylshikimate-3-phosphate synthase (epsps) gene developed to confer glyphosate resistance in rice. Controlling for genetic background, we examined physiological traits and field performance of crop-weed hybrid lineages that segregated for the presence or absence of this novel epsps transgene. Surprisingly, we found that transgenic F2 crop-weed hybrids produced 48-125% more seeds per plant than nontransgenic controls in monoculture- and mixed-planting designs without glyphosate application. Transgenic plants also had greater EPSPS protein levels, tryptophan concentrations, photosynthetic rates, and per cent seed germination compared with nontransgenic controls. Our findings suggest that over-expression of a native rice epsps gene can lead to fitness advantages, even without exposure to glyphosate. We hypothesize that over-expressed epsps may be useful to breeders and, if deployed, could result in fitness benefits in weedy relatives following transgene introgression.

http://www.ncbi.nlm.nih.gov/pubmed/23905647

4 A impossível coexistência

Fluxo gênico por fecundação cruzada (polinização), dispersão de sementes ou ainda THG em cultivos subsequentes a cultivos GM representam fatores naturais[39], fora de controle do agricultor e das empresas de biotecnologia, que impossibilitam a coexistência a campo das plantas GM e não GM.

Ao mesmo tempo em que alguns pesquisadores acreditam na possibilidade de controlar os fatores biológicos envolvidos na contaminação genética (distâncias de isolamento, áreas de reserva, uso de tecnologias GURTs, etc.), outros afirmam que em situações reais o isolamento é impossível e a coexistência sempre implicará fluxo gênico indesejável. Nesse ponto há consenso na comunidade científica quanto à impossibilidade de assegurar contaminação nula nas cadeias agroalimentares convencionais. Os motivos envolvem desde razões biológicas até elementos de natureza socioeconômica associados à expansão do controle das sementes e à acumulação de royalties/patentes sobre o uso de tecnologias registradas.

Devos, Y. ; Reheul, D. ; Schrijver, A. ; Cors, F. ; Moens, W. 2004. Management of herbicide-tolerant oilseed rape in Europe: a case study on minimizing vertical gene flow. *Environ Biosafety Res.,* 3(3):135-48.

The potential commercialization of genetically modified herbicide-tolerant (GMHT) oilseed rape in Europe raises various concerns about their potential environmental and agronomic impacts, especially those associated with the escape of transgenes. Pollen of oilseed rape can be dispersed in space, resulting in the fertilization of sympatric compatible wild relatives (e.g. Brassica rapa) and oilseed rape cultivars grown nearby (GM and/or non-GM Brassica napus). The spatial and temporal dispersal of seeds of oilseed rape may lead to feral oilseed rape populations outside the

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[39] Confira com item 3 da Parte 3 para mais referências sobre fluxo gênico entre espécies aparentadas não agrícolas.

cropped areas and oilseed rape volunteers in subsequent crops in the rotation. The incorporation of a HT trait(s) may increase the fitness of the recipient plants, making them more abundant and persistent, and may result in weeds that are difficult to control by the herbicide(s) to which they are tolerant. Vertical gene flow from transgenic oilseed rape to non-GM counterparts may also have an impact on farming and supply chain management, depending on labelling thresholds for the adventitious presence of GM material in non-GM products. Given the extent of pollen and seed dispersal in oilseed rape, it is obvious that the safe and sound integration of GMHT oilseed rape in Europe may require significant on-farm and off-farm management efforts. Crucial practical measures that can reduce vertical gene flow include (1) isolating seed production of Brassica napus, (2) the use of certified seed, (3) isolating fields of GM oilseed rape, (4) harvesting at the correct crop development stage with properly adjusted combine settings, (5) ensuring maximum germination of shed seeds after harvest, (6) controlling volunteers in subsequent crops, and (7) keeping on-farm records. The implementation of the recommended practices may, however, be difficult, entailing various challenges.

http://www.ncbi.nlm.nih.gov/pubmed/15901096

Hofmann, F.; Otto, M.; Wosniok, W. 2014. Maize pollen deposition in relation to distance from the nearest pollen source under common cultivation - results of 10 years of monitoring (2001 to 2010). *Environmental Sciences Europe,* 26:24.

Background: Information on pollen dispersal is essential for the risk assessment and management of genetically modified organisms (GMOs) such as Bt maize. We analyzed data on maize pollen deposition at 216 sites in Germany, Switzerland, and Belgium from 2001 to 2010. All data were collected using the same standardized sampling method. The distances between sampling site and the nearest maize field ranged from within the field to 4.45 km.
Results: Maize pollen deposition was negatively correlated with distance from the nearest pollen source. The highest pollen deposition was within the field, but depositions of several thousand pollen grains per square meter were recorded over the kilometer range. A power function model most accurately described the relationship between deposition and distance from the nearest pollen source, rather than the exponential model currently used in EU risk assessment and management, which underestimates exposure for distances greater than 10 m. Regression analysis confirmed the high significance of the power relationship. The large variation in pollen deposition at a given distance reflected the influences of wind direction and other meteorological and site conditions. Plausible variations of single values and the predicted mean pollen count at a given distance were expressed by confidence intervals.
Conclusions: The model described here allows estimations of pollen deposition in relation to distance from the nearest field; therefore, it will be valuable for the risk assessment and management of GMOs. Our results indicate that buffer zones in the kilometer range are required to prevent harmful exposure of non-target organisms to GMOs.

Artigo completo disponível em http://www.enveurope.com/content/26/1/24

REV – Price, B.; Cotter, J. 2014. The GM Contamination Register: a review of recorded contamination incidents associated with genetically modified organisms (GMOs), 1997–2013. *International Journal of Food Contamination,* 1:5.

Background: Since large-scale commercial planting of genetically modified (GM) crops began in 1996, a concern has been that non-GM crops may become contaminated by GM crops and that wild or weedy relatives of GM crops growing outside of cultivated areas could become contaminated. The GM Contamination Register contains records of GM contamination incidents since 1997 and forms a unique database. By the end of 2013, 396 incidents across 63 countries had been recorded.
Results: Analysis of the Register database reveals rice has the highest number of GM contamination

incidents of all crops (accounting for a third of incidents), despite there being no commercial growing of GM rice anywhere in the world. The majority of these incidents derive from two distinct cases of contamination of unauthorised GM rice lines, LLRICE from the USA and BT63 rice from China. Maize accounts for 25% of GM contamination incidents, whilst soya and oilseed rape account for approximately 10% of incidents. Although factors such as acreage grown, plant biology, designation as a food or non food crop and degree of international trading can potentially affect the frequency and extent of contamination, it is not possible to determine which are dominant. The Register records a total of nine cases of contamination from unauthorised GM lines, i.e. those at the research and development stage with no authorisation for commercial cultivation anywhere in the world. An important conclusion of this work is that GM contamination can occur independently of commercialisation. Some of these cases, notably papaya in Thailand, maize in Mexico and grass in USA have continued over a number of years and are ongoing, whilst other contamination cases such as Bt10 maize and pharmaceutical-producing GM crops occur only with a single year. The route(s) of contamination are often unclear.

Conclusions: The detection of GMO contamination is dependent on both routine and targeted monitoring regimes, which appears to be inconsistent from country to country, even within the EU. The lack of an analytical methodology for the detection of GM crops at the field trial stage (i.e. pre-commercialisation) can hamper efforts to detect any contamination arising from such GM lines.

Artigo completo disponível em http://link.springer.com/article/10.1186%2Fs40550-014-0005-8

4.1 Contaminação de cultivos não GM por GM, via polinização

Em função da espécie, das condições climáticas e do mosaico de lavouras considerados, o fluxo gênico por polinização pode ocorrer entre indivíduos separados por grandes distâncias. Cada espécie apresentará suas particularidades no que diz respeito ao potencial de contaminação por fecundação cruzada. Entretanto, o histórico de contaminações ocorridas ao redor do mundo mostra que nenhuma variedade convencional que possua equivalentes transgênicas estará protegida contra o fluxo gênico por polinização. Além disso, existem outros fatores relacionados ao comportamento humano que ampliam os riscos de contaminação.

Rieger, M.; Lamond, M.; Preston, C.; Powles, S.; Roush, R. 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*, 296 (5577): 2386-2388.

There is considerable public and scientific debate for and against genetically modified (GM) crops. One of the first GM crops, *Brassica napus* (oilseed rape or canola) is now widely grown in North America, with proposed commercial release into Australia and Europe. Among concerns of opponents to these crops are claims that pollen movement will cause unacceptable levels of gene flow from GM to non-GM crops or to related weedy species, resulting in genetic pollution of the environment. Therefore, quantifying pollen-mediated gene flow is vital for assessing the environmental impact of GM crops. This study quantifies at a landscape level the gene flow that occurs from herbicide-resistant canola crops to nearby crops not containing herbicide resistance genes.

Artigo completo disponível em http://www.sciencemag.org/content/296/5577/2386.long

Watrud, L.; Lee, E.; Fairbrother, A.; Burdick, C.; Reichman, J.; Bollman, M.; Storm, M.; King, G.; Van de Water, P. 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *PNAS*, vol. 101 (40). 14533-14538.

Sampling methods and results of a gene flow study are described that will be of interest to plant scientists, evolutionary biologists, ecologists, and stakeholders assessing the environmental safety of transgenic crops. This study documents gene flow on a landscape level from creeping bentgrass (*Agrostis stolonifera* L.), one of the first wind-pollinated, perennial, and highly outcrossing transgenic crops being developed for commercial use. Most of the gene flow occurred within 2 km in the direction of prevailing winds. The maximal gene flow distances observed were 21 km and 14 km in sentinel and resident plants, respectively, that were located in primarily nonagronomic habitats. The selectable marker used in these studies was the *CP4 EPSPS* gene derived from *Agrobacterium* spp. strain CP4 that encodes 5-enol-pyruvylshikimate-3-phosphate synthase and confers resistance to glyphosate herbicide. Evidence for gene flow to 75 of 138 sentinel plants of *A. stolonifera* and to 29 of 69 resident *Agrostis* plants was based on seedling progeny survival after spraying with glyphosate in greenhouse assays and positive TraitChek, PCR, and sequencing results. Additional studies are needed to determine whether introgression will occur and whether it will affect the ecological fitness of progeny or the structure of plant communities in which transgenic progeny may become established.

Artigo completo disponível em http://www.pnas.org/content/101/40/14533.long

Galeano, P.; Debat, C.; Ruibal, F.; Fraguas, L.; Galván, G. 2010. Cross-fertilization between genetically modified and nongenetically modified maize crops in Uruguay. *Env. Biosafety Res.,* vol 9 (3): 147-154.

The cultivation of transgenic (GM) Bt maize (events Mon810 and Bt11) is permitted in Uruguay. Local regulations ensures that 10% of the crop should be a non-GM cultivar as reservation area for biodiversity, and the distance from other non-GM maize crops should be more than 250 m in order to avoid cross-pollination. However, the degree of cross-pollination between maize crops in Uruguay is unknown. The level of contamination with Bt is a relevant issue for organic farming, *in-situ* conservation of genetic resources, and seed production. In this research the occurrence and frequency of cross-pollination between commercial GM and non-GM maize crops in Uruguay was assessed. The methodology comprised field sampling and detection using DAS-ELISA and PCR. Five field-pair situations of GM and non-GM nearly grown with potential risk of pollen contamination were identified, regarding the distance between crops and similarity between sowing dates. Among these five situations, Bt contamination was found in three cases. The detected transgenic events were coincident with the event in each potential source of contamination. Frequencies of transgenic events in the offspring of non-GM crops were estimated as 1/60, 1/40 and 1/255 for three field situations with distances of respectively 40, 100 and 330 m from the source of contamination. This is a first indication that GM contamination may frequently occur in Uruguay if isolation by distance and/or time is not provided. These findings contribute to evaluate the applicability of regulated co-existence policy.

http://www.ncbi.nlm.nih.gov/pubmed/21975255

4.2 Disseminação de transgenes por meio de populações ferais

Em função das espécies, o escape de transgenes em populações de plantas ruderais associadas a lavouras comerciais transgênicas (ou adjacentes a elas) se dá – e apresenta probabilidade de permanecer no tempo – em graus variáveis. Mas quando isso ocorre, e para além dos riscos ambientais assim gerados, essas populações viram reservatórios de transgenes que podem ampliar sua disseminação, seja por meio de pólen seja por sementes. Isso alcança mesmo lavouras convencionais relativamente isoladas – gerando problemas de coexistência e de relacionamento entre vizinhos.

Ellstrand, N. 2001. When transgenes wander, should we worry? *Plant Physiol.*, 125:1543–1545.

Sem resumo.

Artigo completo disponível em http://www.plantphysiol.org/content/125/4/1543.full.pdf+html

Simard, M.; Legere, A.; Warwick, S. 2006. Transgenic *Brassica napus* fields and *Brassica rapa* weeds in Quebec sympatry and weed-crop in situ hybridization. *Canadian Journal of Botany,* 84: 1842-1851.

Hybridization between the herbicide-resistant transgenic crop Brassica napus L. (canola) and its weedy relative Brassica rapa L. (bird rape) has been documented in Quebec. Our goal was to evaluate the actual hybridization potential based on range overlap and actual in situ hybridization rates. This was done by mapping *B. napus* canola fields, comparing them with the sampling locations of *B. rapa* herbarium specimens from Quebec, gathering information on the presence of *B. rapa* in certified canola seed production fields, and surveying for *B. rapa* populations located in, or close to *B. napus* field margins. Progeny from these populations were screened for herbicide resistance (HR) and for the presence of the HR transgene. Two fields were also selected to evaluate *B. rapa* density effects on hybridization rates. Significant sympatry was observed in several areas of the province; hybridization occurred in all eight populations (1.1% to 17.5% hybrid seed) located in field margins and in one (1.1%) out of three populations located less than 10 m from a *B. napus* field. Hybridization rates decreased exponentially as *B. rapa* density increased, but interplant rates (0% to 68%) were highly variable. Environmental problems could be generated by the release of *B. napus* crops with traits conferring fitness benefits in nonmanaged areas.

http://www.nrcresearchpress.com/doi/abs/10.1139/b06-135#.VMJ34fldWt8

Knispel, A.; McLachlan, S.; Van Acker, R.; Friesen, L. 2008. Gene flow and multiple herbicide resistance in escaped canola populations. *WeedScience*, 56, 72-80.

Gene flow among herbicide-resistant (HR) canola varieties can lead to the development of multiple HR canola plants, creating volunteer canola management challenges for producers. In western Canada, escaped populations of HR canola are ubiquitous outside of cultivated fields, yet the extent of gene flow resulting in herbicide resistance trait stacking in individuals within these populations remains unknown. The objectives of this study were to document the presence of single and multiple herbicide resistance traits and assess the extent of gene flow within escaped canola populations. Seed was collected from 16 escaped canola populations along the verges of fields and roadways in four agricultural regions in southern Manitoba from 2004 to 2006. Glyphosate resistance was found in 14 (88%) of these populations, glufosinate resistance in 13 (81%) populations, and imidazolinone resistance in five (31%) populations. Multiple herbicide resistance was observed at levels consistent with previously published canola outcrossing rates in 10 (62%) of the tested populations. In 2005 and 2006, maternal plants from two escaped populations were tested using trait indicator test strips for glyphosate and glufosinate resistance to confirm outcrossing events. In 2005, two of 13 tested maternal plants with single herbicide resistance traits produced progeny with both glyphosate and glufosinate resistance. In 2006, of 21 tested plants, 10 single HR maternal plants produced multiple HR progeny, and five nonresistant maternal plants produced resistant offspring. This is the first report indicating that intraspecific gene flow results in stacking of herbicide resistance traits in individuals within escaped canola populations, confirming that multiple HR canola volunteers are not confined to agricultural fields. Results of this study suggest that escaped populations of crop plants can contribute to the spread of genetically engineered novel traits, which has important implications for containment, especially for highly controversial pharmaceutical and industrial traits in crop plants.

http://www.bioone.org/doi/abs/10.1614/WS-07-097.1?journalCode=wees

Knispel A.; McLachlan, S. 2010. Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada*. Environ Sci Pollut Res Int.*, 17(1):13-25. doi: 10.1007/s11356-009-0219-0.

Background, Aim and Scope: Genetically modified herbicide-tolerant (GMHT) oilseed rape (OSR; Brassica napus L.) was approved for commercial cultivation in Canada in 1995 and currently represents over 95% of the OSR grown in western Canada. After a decade of widespread cultivation, GMHT volunteers represent an increasing management problem in cultivated fields and are ubiquitous in adjacent ruderal habitats, where they contribute to the spread of transgenes. However, few studies have considered escaped GMHT OSR populations in North America, and even fewer have been conducted at large spatial scales (i.e. landscape scales). In particular, the contribution of landscape structure and large-scale anthropogenic dispersal processes to the persistence and spread of escaped GMHT OSR remains poorly understood. We conducted a multi-year survey of the landscape-scale distribution of escaped OSR plants adjacent to roads and cultivated fields. Our objective was to examine the long-term dynamics of escaped OSR at large spatial scales and to assess the relative importance of landscape and localised factors to the persistence and spread of these plants outside of cultivation.
Materials and Methods: From 2005 to 2007, we surveyed escaped OSR plants along roadsides and field edges at 12 locations in three agricultural landscapes in southern Manitoba where GMHT OSR is widely grown. Data were analysed to examine temporal changes at large spatial scales and to determine factors affecting the distribution of escaped OSR plants in roadside and field edge habitats within agricultural landscapes. Additionally, we assessed the potential for seed dispersal between escaped populations by comparing the relative spatial distribution of roadside and field edge OSR.
Results: Densities of escaped OSR fluctuated over space and time in both roadside and field edge habitats, though the proportion of GMHT plants was high (93-100%). Escaped OSR was positively affected by agricultural landscape (indicative of cropping intensity) and by the presence of an adjacent field planted to OSR. Within roadside habitats, escaped OSR was also strongly associated with large-scale variables, including road surface (indicative of traffic intensity) and distance to the nearest grain elevator. Conversely, within field edges, OSR density was affected by localised crop management practices such as mowing, soil disturbance and herbicide application. Despite the proximity of roadsides and field edges, there was little evidence of spatial aggregation among escaped

OSR populations in these two habitats, especially at very fine spatial scales (i.e. <100 m), suggesting that natural propagule exchange is infrequent.
Discussion: Escaped OSR populations were persistent at large spatial and temporal scales, and low density in a given landscape or year was not indicative of overall extinction. As a result of ongoing cultivation and transport of OSR crops, escaped GMHT traits will likely remain predominant in agricultural landscapes. While escaped OSR in field edge habitats generally results from local seeding and management activities occurring at the field-scale, distribution patterns within roadside habitats are determined in large part by seed transport occurring at the landscape scale and at even larger regional scales. Our findings suggest that these large-scale anthropogenic dispersal processes are sufficient to enable persistence despite limited natural seed dispersal. This widespread dispersal is likely to undermine field-scale management practices aimed at eliminating escaped and in-field GMHT OSR populations.
Conclusions: Agricultural transport and landscape-scale cropping patterns are important determinants of the distribution of escaped GM crops. At the regional level, these factors ensure ongoing establishment and spread of escaped GMHT OSR despite limited local seed dispersal. Escaped populations thus play an important role in the spread of transgenes and have substantial implications for the coexistence of GM and non-GM production systems.
Recommendations and Perspectives: Given the large-scale factors driving the spread of escaped transgenes, localised co-existence measures may be impracticable where they are not commensurate with regional dispersal mechanisms. To be effective, strategies aimed at reducing contamination from GM crops should be multi-scale in approach and be developed and implemented at both farm and landscape levels of organisation. Multiple stakeholders should thus be consulted, including both GM and non-GM farmers, as well as seed developers, processors, transporters and suppliers. Decisions to adopt GM crops require thoughtful and inclusive consideration of the risks and responsibilities inherent in this new technology.

http://www.ncbi.nlm.nih.gov/pubmed/19588180

Heuberger, S.; Ellers-Kirk, C.; Tabashnik, B.; Carrière, Y. 2010. Pollen- and Seed-Mediated Transgene Flow in Commercial Cotton Seed Production Fields. *PLOS ONE,* 5(11): e14128.

Background: Characterizing the spatial patterns of gene flow from transgenic crops is challenging, making it difficult to design containment strategies for markets that regulate the adventitious presence of transgenes. Insecticidal *Bacillus thuringiensis* (Bt) cotton is planted on millions of hectares annually and is a potential source of transgene flow.
Methodology/Principal Findings: Here we monitored 15 non-Bt cotton (*Gossypium hirsutum*, L.) seed production fields (some transgenic for herbicide resistance, some not) for gene flow of the Bt cotton *cry1Ac* transgene. We investigated seed-mediated gene flow, which yields adventitious Bt cotton plants, and pollen-mediated gene flow, which generates outcrossed seeds. A spatially-explicit statistical analysis was used to quantify the effects of nearby Bt and non-Bt cotton fields at various spatial scales, along with the effects of pollinator abundance and adventitious Bt plants in fields, on pollen-mediated gene flow. Adventitious Bt cotton plants, resulting from seed bags and planting error, comprised over 15% of plants sampled from the edges of three seed production fields. In contrast, pollen-mediated gene flow affected less than 1% of the seed sampled from field edges. Variation in outcrossing was better explained by the area of Bt cotton fields within 750 m of the seed production fields than by the area of Bt cotton within larger or smaller spatial scales. Variation in outcrossing was also positively associated with the abundance of honey bees.
Conclusions/Significance: A comparison of statistical methods showed that our spatially-explicit analysis was more powerful for understanding the effects of surrounding fields than customary models based on distance. Given the low rates of pollen-mediated gene flow observed in this study, we conclude that careful planting and screening of seeds could be more important than field spacing for limiting gene flow.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0014128

Park, K.; Lee, B.; Kim, C.; Kim, D.; Park, J.; Ko, E.; Jeong, S.; Choi, K.; Yoon, W.; Kim, H. 2010. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. *Food Control*, 21(4): 456-461.

The cultivation area of genetically modified (GM) crops is increasing all over the world. Though no land in the Republic of Korea is currently used for the cultivation of GM crops, GM crop imports for food and foraging purposes are continuously increasing. This may promote the unintentional escape of GM crops. This study was conducted to investigate whether imported GM maize is released into our environment during the transportation of grain in the Republic of Korea. Based on PCR analysis, most of the maize grains in the forage products were GM, and about 50% of the grains were germinated. Monitoring was conducted in two major grain receiving ports, 15 feed manufacturing plants, and 14 livestock barns in five provinces of the Republic of Korea from July to September 2007. We found many spilled maize grains around open storage areas of ports and along truck transportation routes near feed manufacturing plants. Established maize plants were not found at or around Incheon port. However, we found 18 established maize plants at the Gunsan port, 15 of which were GM. We also found eight GM maize plants around four feed manufacturing plants and in two livestock barns. Based on the event-specific PCR analysis, three maize events (NK603, Mon810, and TC1507) were identified. Though several GM maize plants were found around the port and feed manufacturing plants, most of these facilities were located inside the industrial park and were far from cultivated fields, likely rendering the impact of these GM maize on the natural environments negligible. However, most of the livestock barns were close to cultivated areas. Moreover, maize plants were cultivated for food or feed near some livestock barns. This practice may facilitate gene flow from GM maize to non-GM maize plants. Therefore, continuous monitoring is necessary to detect the occurrence of GM maize, and appropriate action should be taken to prevent genetic admixture in our environment.

http://www.researchgate.net/publication/223384520_Monitoring_the_occurrence_of_genetically_modified_maize_at_a_grain_receiving_port_and_along_transportation_routes_in_the_Republic_of_Korea

Schafer, M.; Ross, A.; Londo, J.; Burdick, C.; Lee, E.; *et al*. 2011. The Establishment of Genetically Engineered Canola Populations in the U.S. *PLOS ONE*, 6(10): e25736. doi:10.1371/journal.pone.0025736.

Concerns regarding the commercial release of genetically engineered (GE) crops include naturalization, introgression to sexually compatible relatives and the transfer of beneficial traits to native and weedy species through hybridization. To date there have been few documented reports of escape leading some researchers to question the environmental risks of biotech products. In this study we conducted a systematic roadside survey of canola (*Brassica napus)* populations growing outside of cultivation in North Dakota, USA, the dominant canola growing region in the U.S. We document the presence of two escaped, transgenic genotypes, as well as non-GE canola, and provide evidence of novel combinations of transgenic forms in the wild. Our results demonstrate that feral populations are large and widespread. Moreover, flowering times of escaped populations, as well as the fertile condition of the majority of collections suggest that these populations are established and persistent outside of cultivation.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0025736

Schoenenberger, N.; D'Andrea, L. 2012. Surveying the occurrence of subspontaneous glyphosate tolerant genetically engineered *Brassica napu*s L. (Brassicaceae) along Swiss railways. *Environmental Sciences Europe*, 24:23.

Background: Railway tracks represent a highly interlinked habitat with numerous possibilities for accidental entry of oilseed rape due to seed spill during transportation. Moreover, glyphosate is regularly employed to control the vegetation, increasing the possibility of establishment for plants resistant to it. We surveyed the presence of genetically engineered glyphosate tolerant oilseed rape (*Brassica napus*) with a focus on the most important Swiss railway stations. Our objective was to detect accidental establishment of transgenic plants, since Switzerland does not import nor cultivate transgenic oilseed rape.

Results: Seventy-nine railway areas were sampled in Switzerland and the Principality of Liechtenstein, and the feral presence of oilseed rape was detected in 58 of them. A total of 2403 individuals were tested for genetic modification using commercially available immunologic test kits. In four localities, one located in Lugano and three in the area of Basel, a total of 50 plants expressing the CP4 EPSPS protein were detected. In two of the localities, survival of herbicide applications was observed. The populations were probably introduced through contaminated seed spills from freight trains, or during the transfer of goods from cargo ships to trains.

Conclusions: Railways represent an ideal system for herbicide resistant transgenic plants to establish and spread as a result of high selective pressure in favour of herbicide resistance with consequent increased difficulties to keep the infrastructure free of weeds. Crop-to-wild gene flow can occur as several sexually compatible species which are congeneric or in allied genera to oilseed rape were found growing sympatrically. Moreover, the capillary presence of railways in the agricultural landscape provides a putative source of contamination of GE-free agriculture. Our results suggests that carefully adapted monitoring designs may be set in order to detect introduction events that can lead to rapid establishment and growing populations as the accepted contamination thresholds are likely to be biologically insufficient to prevent further environmental contamination.

Artigo completo disponível em http://www.enveurope.com/content/24/1/23

Waminal, N. ; Ryu, K. ; Choi, S.; Kim, H. 2013. Randomly detected genetically modified (GM) maize (*Zea mays L.*) near a transport route revealed a fragile 45S rDNA phenotype. *PLOS ONE*, 8(9):e74060. doi: 10.1371/journal.pone.0074060. eCollection 2013.

Monitoring of genetically modified (GM) crops has been emphasized to prevent their potential effects on the environment and human health. Monitoring of the inadvertent dispersal of transgenic maize in several fields and transport routes in Korea was carried out by qualitative multiplex PCR, and molecular analyses were conducted to identify the events of the collected GM maize. Cytogenetic investigations through fluorescence in situ hybridization (FISH) of the GM maize were performed to check for possible changes in the 45S rDNA cluster because this cluster was reported to be sensitive to replication and transcription stress. Three GM maize kernels were collected from a transport route near Incheon port, Korea, and each was found to contain NK603, stacked MON863 x NK603, and stacked NK603 x MON810 inserts, respectively. Cytogenetic analysis of the GM maize containing the stacked NK603 x MON810 insert revealed two normal compact 5S rDNA signals, but the 45S rDNA showed a fragile phenotype, demonstrating a "beads-on-a-string" fragmentation pattern, which seems to be a consequence of genetic modification. Implications of the 45S rDNA cluster fragility in GM maize are also discussed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3767626/

Schulze, J.; Frauenknecht, T.; Brodmann, P.; Bagutti, C. 2014. Unexpected diversity of feral genetically modified oilseed rape (*Brassica napus* L.) despite a cultivation and import ban in Switzerland. *PLOS ONE*, 9(12): e114477.

Despite cultivation and seed import bans of genetically modified (GM) oilseed rape (*Brassica napus* L.), feral GM plants were found growing along railway lines and in port areas at four sites in Switzerland in 2011 and 2012. All GM plants were identified as glyphosate-resistant GM event GT73 (Roundup Ready, Monsanto). The most affected sites were the Rhine port of Basel and the

St. Johann freight railway station in Basel. To assess the distribution and intra- and interspecific outcrossing of GM oilseed rape in more detail, we monitored these two sites in 2013. Leaves and seed pods of feral oilseed rape plants, their possible hybridization partners and putative hybrid plants were sampled in monthly intervals and analysed for the presence of transgenes by real-time PCR. Using flow cytometry, we measured DNA contents of cell nuclei to confirm putative hybrids. In total, 2787 plants were sampled. The presence of GT73 oilseed rape could be confirmed at all previously documented sampling locations and was additionally detected at one new sampling location within the Rhine port. Furthermore, we found the glufosinate-resistant GM events MS8xRF3, MS8 and RF3 (all traded as InVigor, Bayer) at five sampling locations in the Rhine port. To our knowledge, this is the first time that feral MS8xRF3, MS8 or RF3 plants were detected in Europe. Real-time PCR analyses of seeds showed outcrossing of GT73 into two non-GM oilseed rape plants, but no outcrossing of transgenes into related wild species was observed. We found no hybrids between oilseed rape and related species. GM plants most frequently occurred at unloading sites for ships, indicating that ship cargo traffic is the main entry pathway for GM oilseed rape. In the future, it will be of major interest to determine the source of GM oilseed rape seeds.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4252112/

4.3 Contaminação de lavouras convencionais subsequentes ao plantio de variedades transgênicas

Em paralelo aos problemas de coexistência, existem aspectos operacionais decorrentes da emergência de sementes que ficam no solo – e crescem na lavoura subsequente. Sementes transgênicas que caem no terreno durante a colheita de determinada safra germinam na safra seguinte e persistem junto à lavoura subsequente porque toleram o herbicida que supostamente garantiria limpeza da área[40]. Trata-se de problema que dificulta o manejo e reduz a rentabilidade das lavouras, além de que afeta o valor final da safra (maiores índices de impurezas serão detectados no momento de classificação dos grãos).

D'Hertefeldt, T.; Jørgensen, R.; Pettersson, L. 2008. Long term persistence of GM oilseed rape in the seed bank. *Biology Letters,* 4 (3): 314-7.

Coexistence between genetically modified (GM) and non-GM plants is a field of rapid development and considerable controversy. In crops, it is increasingly important to understand and predict the GM volunteer emergence in subsequent non-GM crops. Theoretical models suggest recruitment from the seedbank over extended periods, but empirical evidence matching these predictions

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[40] Esse problema agronômico é muito presente no Brasil, onde a rotação milho-soja representa o sistema de produção dominante nas principais zonas de cultivo (Centro-Oeste). Nos EUA, onde a prática é limitada pelas condições climáticas de inverno, desfavoráveis a plantios sucessivos, o problema é de menor relevância. Ver matéria jornalística elaborada pela Embrapa em <https://www.embrapa.br/busca-de-noticias/-/noticia/1920015/milho-voluntario-rr-pode-se-tornar-planta-daninha-para-soja>.

has been scarce. Here, we provide evidence of long-term GM seed persistence in conventional agriculture. Ten years after a trial of GM herbicide-tolerant oilseed rape, emergent seedlings were collected and tested for herbicide tolerance. Seedlings that survived the glufosinate herbicide (15 out of 38 volunteers) tested positive for at least one GM insert. The resulting density was equivalent to 0.01 plantsm^{-2}, despite complying with volunteer reduction recommendations. These results are important in relation to debating and regulating coexistence of GM and non-GM crops, particularly for planting non-GM crops after GM crops in the same field.

Artigo completo disponível em http://rsbl.royalsocietypublishing.org/content/4/3/314.full

Munier, D. ; Brittan, K. ; Lanini, W. 2012. Seed bank persistence of genetically modified canola in California. *Environ Sci Pollut Res Int.,* 19(6):2281-4.

Introduction: Canola, which is genetically modified (GM) for tolerance to glyphosate, has the potential to become established as a new glyphosate resistant weed, thus reducing the effectiveness of glyphosate.
Methods: Volunteer from dormant canola seeds produced thousands of plants per hectare in the fourth year (2011) following a 2007 crop harvest. This occurred with no additional canola seed production since the 2007 harvest.
Results: Volunteer plants following harvests of annual crops are typically only a problem for the first year after harvest. In California, glyphosate is the core herbicide on over a million hectares of high value row, tree, and vine crops and new glyphosate resistant weeds reduce the effectiveness of glyphosate.
Conclusions: The combination of dormant seed and herbicide resistance makes GM glyphosate-resistant canola a new and difficult California weed which was first observed in the winter of 2009.

Artigo completo disponível em http://wric.ucdavis.edu/PDFs/Seed_bank_persistence_of_genetically_modified_canola.pdf

4.4 Contaminação ao longo da cadeia agroalimentar

Além dos aspectos biológicos, as questões relativas à coexistência entre produtos transgênicos e não transgênicos exigem consideração a aspectos de ordem socioeconômica. Nesse sentido são apresentados estudos que apontam casos de contaminações identificadas ao longo da cadeia agroalimentar, gerando tensões entre os diversos atores e causando prejuízos de difícil mensuração, afetos especialmente a quem não deseja plantar/comercializar/consumir produtos GM.

Friesen, L.; Nelson, A.; Van Acker, R. 2003. Evidence of contamination of pedigreed canola (*Brassica napus*) seed lots in western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal,* 95 (5), 1342-1347.

The objective of this study was to survey pedigreed canola L.) seedlots for contaminating herbicide resistance traits because of complaints from farmers regarding glyphosate [*N*-(phosphonomethyl) glycine]-resistant canola volunteers occurring unexpectedly in their fields at densities and in patterns

that suggested that pollen-mediated gene flow from neighboring fields in previous was not the source of contamination. Twenty-seven unique, commercial certified canola seedlot samples were collected. Glyphosate-resistant seedlot samples were not collected. Canola samples were planted in the field, and when the canola had two to four true leaves, glypho- sate, glufosinate [2-amino-4-(hydroxymethylphosphinyl) butanoic acid], and thifensulfuron {methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2yl) amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate} herbicides were applied. Surviving canola plantswere counted.Of the 27 seedlots, 14 had contamination levels above 0.25% and therefore failed 99.75% cultivar purity guideline for certified canola seed. Three seed lots had glyphosate resistance contamination levels in excess of 2.0%. Unexpected contamination (even at 0.25%) can cause problems for producers that practice direct seeding and depend on glyphosate for nonselective, broad-spectrum weed control. To avoid unexpected problems and costs, it is important that farmers are cognizant of the high probability that pedigreed canola seedlots are cross-contaminated with the various herbicide resistance traits.

Artigo completo disponível em http://kurt-schweizer.ch/home/gentech/RapsKanadaVanAcker.pdf

COM – Macilwain, C. 2005. US launches probe into sales of unapproved transgenic corn. *Nature*, Vol 434, p.423.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/15791213

Binimelis, R. 2008. Coexistence of plants and coexistence of farmers: is an individual choice possible? *Journal of Agricultural and Environmental Ethics*, DOI10.1007/s10806-008-9099-4.

The introduction of genetically modified organisms (GMOs) in Europe has been characterized by controversy. In 2002, the European Union introduced the concept of "coexistence" as a compromise solution that, through the establishment of science-based technical measures, should allow the market to operate freely while reducing policy conflicts on GMOs. However, the concept remains highly contested and the technical measures difficult to apply. This paper presents qualitative research on the conceptualization and implementation of the coexistence framework in two regions of Spain (Catalonia and Aragon), where 42% and 55% of maize was GM in 2006, respectively. In this context, the concept of coexistence and its proposed implementation both fail to resolve previous conflicts and actually work to generate new ones through the individualization of choice and impacts. Considerations of the social conditions in which the technology and the management measures are implemented were not taken into account. This resulted in the promotion of biotechnological agriculture over other alternatives.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/choicebinimelis.pdf

As práticas de trocas de sementes entre agricultores familiares e comunidades tradicionais ao redor do mundo – base de parte significativa da agrobiodiversidade existente – também estão ameaçadas pela livre circulação de variedades transgênicas. Além disto, essas práticas representam um fator de risco para a disseminação involuntária de transgenes sobre variedades locais, tal como foi reportado no México e em vários outros locais do planeta.

Dyer, G.; Serratos-Hernández, J.; Perales, H.; Gepts, P.; Piñeyro-Nelson, A.; *et al*. 2009. Dispersal of Transgenes through Maize Seed Systems in Mexico. *PLOS ONE,* 4(5): e5734. doi:10.1371/journal.pone.0005734.

Objectives: Current models of transgene dispersal focus on gene flow via pollen while neglecting seed, a vital vehicle for gene flow in centers of crop origin and diversity. We analyze the dispersal of maize transgenes via seeds in Mexico, the crop's cradle.
Methods: We use immunoassays (ELISA) to screen for the activity of recombinant proteins in a nationwide sample of farmer seed stocks. We estimate critical parameters of seed population dynamics using household survey data and combine these estimates with analytical results to examine presumed sources and mechanisms of dispersal.
Results: Recombinant proteins Cry1Ab/Ac and CP4/EPSPS were found in 3.1% and 1.8% of samples, respectively. They are most abundant in southeast Mexico but also present in the west-central region. Diffusion of seed and grain imported from the United States might explain the frequency and distribution of transgenes in west-central Mexico but not in the southeast.
Conclusions: Understanding the potential for transgene survival and dispersal should help design methods to regulate the diffusion of germplasm into local seed stocks. Further research is needed on the interactions between formal and informal seed systems and grain markets in centers of crop origin and diversification.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0005734

Iversen, M.; Grønsberg, I.; Van Den Berg, J.; Fischer, K.; Aheto, D.; Bøhn, T. 2014. Detection of transgenes in local maize varieties of small-scale farmers in eastern cape, South Africa. *PLOS ONE*, 9(12):e116147. doi: 10.1371/journal.pone.0116147.

Small-scale subsistence farmers in South Africa have been introduced to genetically modified (GM) crops for more than a decade. Little is known about i) the extent of transgene introgression into locally recycled seed, ii) what short and long-term ecological and socioeconomic impacts such mixing of seeds might have, iii) how the farmers perceive GM crops, and iv) to what degree approval conditions are followed and controlled. This study conducted in the Eastern Cape, South Africa, aims primarily at addressing the first of these issues. We analysed for transgenes in 796 individual maize plants (leaves) and 20 seed batches collected in a village where GM insect resistant maize was previously promoted and grown as part of an governmental agricultural development program over a seven year period (2001-2008). Additionally, we surveyed the varieties of maize grown and the farmers' practices of recycling and sharing of seed in the same community (26 farmers were interviewed). Recycling and sharing of seeds were common in the community and may contribute to spread and persistence of transgenes in maize on a local or regional level. By analysing DNA we found that the commonly used transgene promoter p35s occurred in one of the 796 leaf samples (0.0013%) and in five of the 20 seed samples (25%). Three of the 20 seed samples (15%) included herbicide tolerant maize (NK603) intentionally grown by the farmers from seed bought from local seed retailers or acquired through a currently running agricultural development program. The two remaining positive seed samples (10%) included genes for insect resistance (from MON810). In both cases the farmers were unaware of the transgenes present. In conclusion, we demonstrate that transgenes are mixed into seed storages of small-scale farming communities where recycling and sharing of seeds are common, i.e. spread beyond the control of the formal seed system.

Artigo completo disponível em http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0116147

Parte 3

Riscos para o meio ambiente associados ao cultivo e/ou uso de plantas transgênicas

A síntese de toxinas Bt e a tolerância a herbicidas são as características incorporadas por modificação genética à esmagadora maioria das plantas transgênicas, em estudo e liberadas comercialmente no planeta.

Essas características, que no mundo real também envolvem combinações em eventos piramidados, serão tratadas separadamente neste capítulo.

Os artigos selecionados mostram que, em todos os casos, os estudos disponíveis não permitem sustentar a hipótese de ausência de riscos ambientais. As análises são frágeis, as amostras são insuficientes, os métodos são discutíveis, mas, fundamentalmente, a complexidade das relações ecológicas envolvidas não é coberta pelos delineamentos de pesquisa.

Questões relativas a impactos ambientais devem ser tratadas com base em referenciais adequados e cobrindo o leque de alternativas que se estende desde a ausência das tecnologias até o exame de impactos causados por produtos e pacotes tecnológicos a elas associados. Assim, quando alguns cientistas ou atores políticos preferem comparar impactos ambientais decorrentes do uso das plantas transgênicas com (e apenas com) aqueles gerados em agrossistemas manejados com aporte intensivo de agrotóxicos, deixando de lado manejos amigáveis ao ambiente, de base agroecológica, evidentemente estão optando por não levar em conta o inteiro conjunto de possibilidades reais.

No espírito deste livro, assume-se que a avaliação de impactos ambientais também deve considerar referenciais alternativos, envolvendo agroecossistemas de base agroecológica ou orgânicos. Além de cientificamente justificados, esses referenciais se mostram mais bem ajustados ao tema da sustentabilidade e, portanto, da avaliação comparada de impactos socioambientais decorrentes das tecnologias em questão.

Wolfenbarger, L.; Phifer, P. 2000. The ecological risks and benefits of genetically engineered plants. *Science*, Vol 290.

Discussions of the environmental risks and benefits of adopting genetically engineered organisms are highly polarized between pro- and anti-biotechnology groups, but the current state of our knowledge is frequently overlooked in this debate. A review of existing scientific literature reveals that key experiments on both the environmental risks and benefits are lacking. The complexity of ecological systems presents considerable challenges for experiments to assess the risks and benefits and inevitable uncertainties of genetically engineered plants. Collectively, existing studies emphasize that these can vary spatially, temporally, and according to the trait and cultivar modified.

http://www.sciencemag.org/content/290/5499/2088

1 Riscos para o meio ambiente associados ao uso de plantas Bt

1.1 A especificidade molecular e o modo de ação das proteínas Bt ainda não são totalmente compreendidos

As empresas que desenvolvem e comercializam plantas transgênicas afirmam que a principal vantagem da tecnologia Bt estaria associada à especificidade de ação das proteínas Cry. Alojando-se a sítios e receptores específicos do sistema digestivo de determinados insetos, as Cry1 teriam efeito letal apenas sobre alguns insetos da ordem Lepidoptera, enquanto as Cry3 seriam específicas para coleópteros. Assim, efeitos colaterais e danos a organismos não alvo seriam evitados e as tecnologias Bt comprometeriam apenas as populações de organismos elencados como pragas de lavouras.

A revisão científica revela falência dessa premissa. Amplo conjunto de estudos e informações divergentes atestam que as exceções superam a suposta regra de especificidade.

Não apenas inexiste a regra geral apregoada pelas empresas e outros partidários da tecnologia, como se acumulam registros de impactos sobre organismos supostamente insensíveis a determinadas proteínas Cry.

Os conhecimentos disponíveis mostram que o modo de ação dessas proteínas ainda não é totalmente entendido. Estruturas biológicas presentes no organismo dos insetos parecem não constituir receptores passivos e específicos, contribuindo, ao contrário, de forma relevante para efetividade daquelas toxinas.

Deml, R.; Meise, T.; Dettner, K. 1999. Effects of *Bacillus thuringiensis*δδ-endotoxins on food utilization, growth, and survival of selected phytophagous insects. *Journal of Applied Entomology*, 123, pp. 55-64.

Feeding experiments using the Bacillus thuringiensisδ-endotoxins, CryIA(c) and CryIIIA, were conducted with herbivorous insects from various orders (Lepidoptera, Coleoptera, Homoptera) in the laboratory. The mortality data obtained indicate a species-specific susceptibility of the insects to the toxins whereby the feeding habits of the given animal seem to play a negligible part. An unexpected, severely damaging effect of CryIIIA on caterpillars was established, for the first time. By computing various development and nutritional indices it could be shown that retarded growth of the insects tested may not only be traced back to reduced feeding but also to a decreased utilization of food containing an endotoxin. The insect gut seems to be the site of operation and of storage or complete degradation of the endotoxins because neither in the faeces nor in the haemolymph and fat body, could the toxins and their degradation products, respectively, be detected hitherto by means of gel electrophoreses. An altering effect of the toxins on the gut-microflora pattern is indicated from the first examinations but has to be further confirmed. Finally the applicability of these trials in corresponding examinations of transgenic plants producing B. thuringiensis toxin is dicussed.

http://www.researchgate.net/publication/230002640_Effects_of_Bacillus_thuringiensisendotoxins_on_food_utilization_growth_and_survival_of_selected_phytophagous_insects

Lundgren, J.; Wiedenmann, R. 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (*Coleoptera*: *Coccinellidae*). *Environmental Entomology*, 31, pp. 1213-1218.

Coleomegilla maculata DeGeer is a polyphagous predator that is important for suppressing pest populations in corn. We evaluated the impact of Cry3Bb-expressing transgenic corn pollen (event MON863) on *C. maculata* fitness parameters in the laboratory. *C. maculata* larvae were fed mixtures of pollen containing 0, 25, 50, 75, or 100% transgenic pollen, aphids, or were not fed; and the duration of each instar and pupal weight were compared among treatments. In a second trial, other *C. maculata* larvae were reared on one of the pollen mixtures or artificial diet; and the duration of larval and pupal stages, pupal weight, adult mobility, adult survivorship, and female fecundity were compared among treatments. There were no differences in any of the fitness parameters among *C. maculata* in the treatments fed different mixtures of pollen. Beetles in the pollen mixture treatments had faster larval development times, greater larval survivorship, and greater pupal weight than the beetles fed only aphids or an artificial diet. We conclude that we did not detect any effects on the fitness of *C. maculata* that ingested pollen from event MON863. However, these results do not necessarily apply to other transgenic crops expressing toxins specific to Coleoptera.

http://www.bioone.org/doi/abs/10.1603/0046-225X-31.6.1213?journalCode=enve

Tsuda, Y.; Nakatani, F.; Hashimoto, K.; Ikawa, S.; Matsuura, C.; Fukada, T.; Sugimoto, K.; Himeno, M. 2003. Cytotoxic activity of *Bacillus thuringiensis* Cry proteins on mammalian cells transfected with cadherin-like Cry receptor gene of *Bombyx mori* (silkworm). *Biochemical Journal*, 369, 697-703.

Bacillus thuringiensis is widely used as a biological pesticide to control insects that either cause damage to crops or transmit disease. That it can also target the model organism *Caenorhabditis elegans* has not only provided exciting new insights into how the toxins produced by the bacterium target their victims but also how target insects counter the attack. Modern approaches such as reverse genetics and microarray technology have revealed novel receptors for the toxins and possible signal transduction pathways induced within the host following intoxication. This article will discuss how these findings fit in with current models and how they might influence future studies.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1223121/

Hussein, H.; Habustová, O.; Sehnal, F. 2005. Beetle–specific *Bacillus thuringiensis* Cry3Aa toxin reduces larval growth and curbs reproduction in *Spodoptera littoralis* (Boisd.). *Pest Management Science*, 61, 1186–1192.

Application of Bacillus thuringiensis tenebrionis (Bt) and expression of the Bt protein Cry3Aa in genetically modified crops are used for targeted control of the Colorado potato beetle Leptinotarsa decemlineata (Say). The Cry3A proteins are selectively toxic for the beetles but the present study describes effects of Cry3Aa on the Egyptian armyworm, Spodoptera littoralis (Boisduval). Cry3Aa expressed in potatoes or added to an agar-base wheat-germ diet reduced the growth of S. littoralis caterpillars and the fertility of adults. The effect of 1.4 mg kg(-1) Cry3Aa in potato leaves was comparable with that of 3.3 mg kg(-1) in the diet. This difference in activity was correlated with better digestibility and higher conversion efficiency of the diet that also supported higher reproduction rate: S. littoralis grown on the potatoes reached a similar size to those on the diet but laid only 702 instead of 1077 eggs per female. Cry3Aa consumption reduced body growth as a consequence of lower food intake without significantly affecting food digestibility and the conversion efficiency of nutrients. The 11% and 5% body weight reductions caused by 1.4 mg kg(-1) Cry3Aa in potatoes and 3.3 mg kg(-1) in the diet, respectively, were associated with 74% and 65% reduction in the number of progeny; S littoralis grown on a diet with 9.1 mg kg(-1) Cry3Aa were 10% smaller and produced no viable progeny. These data suggest that the curtailment of reproduction was not caused by a general shortage of nutrient reserves but by a more direct Cry3Aa effect on the reproduction process.

http://www.ncbi.nlm.nih.gov/pubmed/16152673

REV - Crickmore, N. 2005. Using worms to better understand how *Bacillus thuringiensis* kills insects. *Trends in Microbiology*, Vol.13 No.8.

Bacillus thuringiensis is widely used as a biological pesticide to control insects that either cause damage to crops or transmit disease. That it can also target the model organism *Caenorhabditis elegans* has not only provided exciting new insights into how the toxins produced by the bacterium target their victims but also how target insects counter the attack. Modern approaches such as reverse genetics and microarray technology have revealed novel receptors for the toxins and possible signal transduction pathways induced within the host following intoxication. This article will discuss how these findings fit in with current models and how they might influence future studies.

http://www.cell.com/trends/microbiology/abstract/S0966-842X%2805%2900156-3

Hussein, H.; Habustová, O.; Turanli, F.; Sehnal, F. 2006. Potato expressing beetle–specific *Bacillus thuringienis* Cry3Aa toxin reduces performance of a moth. *Journal of Chemical Ecology*, 32, 1–13.

Expression of the Bacillus thuringiensis beetle-specific toxin Cry3Aa, which renders a genetically modified potato cultivar resistant to the Colorado potato beetle Leptinotarsa decemlineata, exerts a deleterious effect on the polyphagous moth Spodoptera littoralis. The caterpillars of S. littoralis feed less and produce smaller pupae on the genetically modified cultivar (NewLeaf Superior) than on the parental nontransgenic cultivar (Superior). The conversion efficiencies of total dry matter, combustion heat, carbon, and nitrogen from leaves to insect biomass are similar on both cultivars. In spite of similar food utilization and a relatively small difference in the body mass at pupation, female adults that developed from caterpillars fed on NewLeaf Superior lay a mean of 309 eggs compared to a mean of 713 eggs deposited by females that developed from caterpillars fed on Superior. Because of this difference and a simultaneous reduction in fertility (egg hatchability) from 78 to 48%, a pair of adults that fed as larvae on NewLeaf Superior produces only 148 larvae, whereas a pair of adults that fed as larvae on Superior produces 556 larvae. We suggest that small amounts of Cry3Aa that accumulate in insect tissue and persist until the adult stage are responsible for the decline in reproduction.

http://www.ncbi.nlm.nih.gov/pubmed/16525866

REV – Hilbeck, A.; Schmidt J. 2006. Another view on Bt proteins - How specific are they and what else might they do? *Biopesticides International*, 2: 1-50.

The entomopathogenic bacterium *Bacillus thuringiensis* (Bt) and its toxins are extensively used for pest control purposes in agriculture, forestry and public health programmes since the 1930. In addition to spray formulations, transgenic plants containing Bt genes for the expression of the toxins (Bt plants) are commercially available since the mid 1990s and are grown on an increasing percentage of the global agricultural area. A main reason for the importance of Bt as a pesticide is the assumed environmental safety concluded from the high specificity of its endotoxins (Cry proteins) towards a limited number of target organisms, mostly distinct groups of pest insects. While the mode of action of the Cry toxins in these susceptible target insects is well studied, Bt experts claim that several details are still not understood well enough. Although there is considerable experience with the application and the environmental safety of Bt sprays, a number of research papers were published in the past that did report adverse effects on non-target organisms. These and the widespread use of transgenic Bt plants stimulated us to review the published laboratory feeding studies on effects of Bt toxins and transgenic Bt plants on non-target invertebrates. We describe those reports that documented adverse effects in non-target organisms in more detail and focus on one prominent example, the green lacewing, *Chrysoperla carnea*. Discussing our findings in the context of current molecular studies, we argue firstly that the evidence for adverse effects in non-target organisms is compelling enough that it would merit more research. We further conclude from our in-depth analysis that the published reports studying the effects of Bt toxins from Bt pesticides and transgenic Bt plants on green lacewing larvae provide complementary and not contradictory data. And, finally, we find that the key experiments explaining the mode of action not only in this particular affected non-target species but also in most other affected non-target species are still missing. Considering the steadily increasing global production area of Bt crops, it seems prudent to thoroughly understand how Bt toxins might affect non-target organisms.

http://www.connectjournals.com/achivestoc.php?bookmark=CJ-023217&volume=2&-year=2006&issue_id=01&issue_month=June#

REV - Bravo, A.; Gill, S.; Soberón, M. 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon*, 49, 423–435.

Bacillus thuringiensis Cry and Cyt protein families are a diverse group of proteins with activity against insects of different orders - Lepidoptera, Coleoptera, Diptera and also against other invertebrates such as nematodes. Their primary action is to lyse midgut epithelial cells by inserting into the target membrane and forming pores. Among this group of proteins, members of the 3-Domain Cry family are used worldwide for insect control, and their mode of action has been characterized in some detail. Phylogenetic analyses established that the diversity of the 3-Domain Cry family evolved by the independent evolution of the three domains and by swapping of domain III among toxins. Like other pore-forming toxins (PFT) that affect mammals, Cry toxins interact with specific receptors located on the host cell surface and are activated by host proteases following receptor binding resulting in the formation of a pre-pore oligomeric structure that is insertion competent. In contrast, Cyt toxins directly interact with membrane lipids and insert into the membrane. Recent evidence suggests that Cyt synergize or overcome resistance to mosquitocidal-Cry proteins by functioning as a Cry-membrane bound receptor. In this review we summarize recent findings on the mode of action of Cry and Cyt toxins, and compare them to the mode of action of other bacterial PFT. Also, we discuss their use in the control of agricultural insect pests and insect vectors of human diseases.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1857359/

Jimenez-Juarrez, N.; Munoz-Garay, C.; G**ó**mez, I.; Saab-Rincon, G.; Damina-Almazo, J.; Gill, S.; Soberón, M.; Bravo, A. 2007. *Bacillus thuringiensis* Cry1Ab mutants affecting oligomer formation are non-toxic to *Manduca sexta* larvae. *The Journal of Biological Chemistry*, 282, pp. 21222-21229.

Pore-forming toxins are biological weapons produced by a variety of living organisms, particularly bacteria but also by insects, reptiles, and invertebrates. These proteins affect the cell membrane of their target, disrupting permeability and leading eventually to cell death. The pore-forming toxins typically transform from soluble, monomeric proteins to oligomers that form transmembrane channels. The Cry toxins produced by Bacillus thuringiensis are widely used as insecticides. These proteins have been recognized as pore-forming toxins, and their primary action is to lyse midgut epithelial cells in their target insect. To exert their toxic effect, a prepore oligomeric intermediate is formed leading finally to membrane-inserted oligomeric pores. To understand the role of Cry oligomeric pre-pore formation in the insecticidal activity we isolated point mutations that affected toxin oligomerization but not their binding with the cadherin-like, Bt-R(1) receptor. We show the helix alpha-3 in domain I contains sequences that could form coiled-coil structures important for oligomerization. Some single point mutants in this helix bound Bt-R(1) receptors with similar affinity as the wild-type toxin, but were affected in oligomerization and were severally impaired in pore formation and toxicity against Manduca sexta larvae. These data indicate the pre-pore oligomer and the toxin pore formation play a major role in the intoxication process of Cry1Ab toxin in insect larvae.

Artigo completo disponível em http://www.jbc.org/content/282/29/21222.long

REV - Pigott, C.; Ellar, D. 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. *Microbiology and Molecular Biology Reviews*, 71 (2): 255–281.

Bacillus thuringiensis produces crystalline protein inclusions with insecticidal or nematocidal properties. These crystal (Cry) proteins determine a particular strain's toxicity profile. Transgenic crops expressing one or more recombinant Cry toxins have become agriculturally important. Individual Cry toxins are usually toxic to only a few species within an order, and receptors on midgut epithelial cells have been shown to be critical determinants of Cry specificity. The best characterized of these receptors have been identified for lepidopterans, and two major receptor classes have emerged: the aminopeptidase N (APN) receptors and the cadherin-like receptors. Currently, 38 different APNs have been reported for 12 different lepidopterans. Each APN belongs to one of five groups that have unique structural features and Cry-binding properties. While 17 different APNs have been reported to bind to Cry toxins, only 2 have been shown to mediate toxin susceptibly in vivo. In contrast, several cadherin-like proteins bind to Cry toxins and confer toxin susceptibility in vitro, and disruption of the cadherin gene has been associated with toxin resistance. Nonetheless, only a small subset of the lepidopteran-specific Cry toxins has been shown to interact with cadherin-like proteins. This review analyzes the interactions between Cry toxins and their receptors, focusing on the identification and validation of receptors, the molecular basis for receptor recognition, the role of the receptor in resistant insects, and proposed models to explain the sequence of events at the cell surface by which receptor binding leads to cell death.

http://www.ncbi.nlm.nih.gov/pubmed/17554045

Schmidt, J.; Braun, C.; Whitehouse, L.; Hilbeck, A. 2009. Effects of activated Bt transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird *Adalia bipunctata* in Laboratory Ecotoxicity Testing. *Arch Environ Contam Toxicol*, 56:221–228.

Insect-active Bacillus thuringiensis (Bt) proteins are expressed by several transgenic crop plants to control certain pests, but nontarget organisms such as ladybirds also can be exposed to these proteins in the field. We developed an improved ecotoxicity testing protocol and conducted feeding trials in a laboratory setting to test for possible adverse effects of different concentrations of microbially produced trypsin-activated Cry1Ab and Cry3Bb toxins on the coccinellid Adalia bipunctata. Larval/pupal mortality, development time, and overall body mass accumulation were recorded. Even at the lowest concentration (5 microg/ml), A. bipunctata larvae fed with the lepidopteran-active Cry1Ab toxin exhibited significantly higher mortality than the control group. In experiments with the coleopteran-active Cry3Bb, only a concentration of 25 microg/ml resulted in a marginally significantly higher mortality compared to the control. Both experiments revealed a slight decline in mortality at the highest concentration of 50 microg/ml, though this was statistically significant only in the Cry1Ab treatment. No differences were detected for development time and body mass of newly emerged adults. Dilutions of the expression vector pBD10--used as a control to exclude effects of the toxin production method--at concentrations between 10 and 100 microg/ml revealed no significant effects on either of the studied parameters. This suggests that the increased mortality of larvae in the toxin feeding trials was caused directly by the activated Bt toxins and raises questions regarding their commonly postulated specificity and their mode of action in A. bipunctata. Implications of the reported results for ladybird populations and their biological pest control functions in transgenic crop ecosystems are discussed.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/cry3Bbschmidt.pdf

REV - Soberón, M.; Gill, S.; Bravo, A. 2009. Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells? *Cellular and Molecular Life Science*, 66: 1337-1349.

Cry proteins, produced by Bacillus thuringiensis (Bt), are widely used for the control of insect pests in agriculture as spray products or expressed in transgenic crops, such as maize and cotton. Little was known regarding the mechanism of action of these toxins when the first commercial Bt product was introduced fifty years ago. However, research on the mechanism of action over the last two decades has enhanced our knowledge of toxin interaction with membrane receptors and their effects in insect midgut cells. All this information allowed for the rational design of improved toxins with higher toxicity or toxins that overcome insect resistance, which could compromise Bt use and effectiveness in the field. In this review we discuss and evaluate the different models of the mode of action of Cry toxins, including a discussion about the role of various receptors in toxin action.

http://www.ncbi.nlm.nih.gov/pubmed/19132293

A bibliografia também aponta sinergias e interações entre as diversas proteínas Cry, bem como destas com outras toxinas naturalmente sintetizadas por *Bacillus thuringiensis*.

Pérez, C.; Fernandez, L.; Sun, J.; Folch, J.; Gill, S.; Soberón, M.; Bravo, A. 2005. *Bacillus thuringiensis* subsp. israelensis Cyt1Aa synergizes Cry11Aa toxin by functioning as a membrane-bound receptor. *PNAS*, 102(51):18303–18308.

Bacillus thuringiensis subsp. israelensis produces crystal proteins, Cry (4Aa, 4Ba, 10Aa, and 11Aa) and Cyt (1Aa and 2Ba) proteins, toxic to mosquito vectors of human diseases. Cyt1Aa overcomes insect resistance to Cry11Aa and Cry4 toxins and synergizes the toxicity of these toxins. However, the molecular mechanism of synergism remains unsolved. Here, we provide evidence that Cyt1Aa functions as a receptor of Cry11Aa. Sequential-binding analysis of Cyt1Aa and Cry11Aa revealed that Cyt1Aa binding to Aedes aegypti brush border membrane vesicles enhanced the binding of biotinylated-Cry11Aa. The Cyt1Aa- and Cry11Aa-binding epitopes were mapped by means of the yeast two-hybrid system, peptide arrays, and heterologous competition assays with synthetic peptides. Two exposed regions in Cyt1Aa, loop beta6-alphaE and part of beta7, bind Cry11Aa. On the other side, Cry11Aa binds Cyt1Aa proteins by means of domain II-loop alpha8 and beta-4, which are also involved in midgut receptor interaction. Characterization of single-point mutations in Cry11Aa and Cyt1Aa revealed key Cry11Aa (S259 and E266) and Cyt1Aa (K198, E204 and K225) residues involved in the interaction of both proteins and in synergism. Additionally, a Cyt1Aa loop beta6-alphaE mutant (K198A) with enhanced synergism to Cry11Aa was isolated. Data provided here strongly indicates that Cyt1Aa synergizes or suppresses resistance to Cry11Aa toxin by functioning as a membrane-bound receptor. Bacillus thuringiensis subsp. israelensis is a highly effective pathogenic bacterium because it produces a toxin and also its functional receptor, promoting toxin binding to the target membrane and causing toxicity.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1317914/

REV – Then, C. 2010. Risk assessment of toxins derived from *Bacillus thuringiensis* - synergism, efficacy, and selectivity. *Environ Sci Pollut Res Int*, 17:791-797.

Background, aim, and scope: This review deals with publications concerning the mode of action of Bt proteins and their potential synergism with extrinsic factors. The aim was to assess the impact of those factors especially regarding selectivity and efficacy of Bt toxins and to discuss possible gaps in current risk assessment of genetically engineered plants expressing Bt toxins.Main features: The review shows that several extrinsic factors are able to influence the selectivity and efficacy of Bt toxins. The findings are seen as being relevant for risk assessment in Bt plants. This conclusion is derived by discussing current state of knowledge about the mode of action of Bt proteins, unexpected effects on non-target organism, and the way how modified Bt toxins are expressed in genetically engineered plants.Results: Several publications have been identified that show that certain factors and synergism can impact efficacy and selectivity of Bt toxins. These extrinsic factors are various and include other Bt toxins or parts from the spore of *Bacillus thuringiensis* as well as certain enzymes, environmental stress, non-pathogenic microorganisms, and infectious diseases.Discussion: Research on the underlying mechanism of observed synergism might help to explain some of the effects found in non-target organisms. In general, possible synergism of Bt toxins with extrinsic factors can be relevant for risk assessment of genetically engineered Bt plants since they expose a modified Bt toxin to the environment under various conditions and over a long period of time.Conclusions: Risk assessment of genetically engineered plants should put into question the general assumption of a high selectivity and a linear dose–response relationship in the toxicity of Bt proteins. Both selectivity and efficacy can be influenced by synergism, which can provoke unexpected and undesired effects in non-target organisms.Perspectives: It is suggested that systematic research be promoted on synergism between Bt toxins and potential extrinsic factors that could impact the spectrum of susceptible organisms. This research should become a prerequisite for risk assessment of Bt plants.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2822905/pdf/11356_2009_Article_208.pdf

REV – Soberón, M.; Pardo, L.; Muñóz-Garay, C.; Sánchez, J.; Gómez, I.; Porta, H.; Bravo, A. 2010. Pore formation by Cry toxins. *Advances in Experimental Medicine and Biology*, 677:127-42.

Bacillus thuringiensis (Bt) bacteria produce insecticidal Cry and Cyt proteins used in the biological control of different insect pests. In this review, we will focus on the 3d-Cry toxins that represent the biggest group of Cry proteins and also on Cyt toxins. The 3d-Cry toxins are pore-forming toxins that induce cell death by forming ionic pores into the membrane of the midgut epithelial cells in their target insect. The initial steps in the mode of action include ingestion of the protoxin, activation by midgut proteases to produce the toxin fragment and the interaction with the primary cadherin receptor. The interaction of the monomeric CrylA toxin with the cadherin receptor promotes an extra proteolytic cleavage, where helix alpha-1 of domain I is eliminated and the toxin oligomerization is induced, forming a structure of 250 kDa. The oligomeric structure binds to a secondary receptor, aminopeptidase N or alkaline phosphatase. The secondary receptor drives the toxin into detergent resistant membrane microdomains formingpores that cause osmotic shock, burst of the midgut cells and insect death. Regarding to Cyt toxins, these proteins have a synergistic effect on the toxicity of some Cry toxins. Cyt proteins are also proteolytic activated in the midgut lumen of their target, they bind to some phospholipids present in the mosquito midgut cells. The proposed mechanism of synergism between Cry and Cyt toxins is that Cyt1Aa function as a receptor for Cry toxins. The Cyt1A inserts into midgut epithelium membrane and exposes protein regions that are recognized by Cry11Aa. It was demonstrated that this interaction facilitates the oligomerization of Cry11Aa and also its pore formation activity.

http://www.ncbi.nlm.nih.gov/pubmed/20687486

1.2 Impactos negativos da tecnologia Bt sobre Organismos Não Alvo (ONAs)

Incertezas relativas ao modo de ação das toxinas Cry recomendam avaliação cuidadosa de impactos sobre os chamados “organismos não alvo” (ONAs). Representando um vasto conjunto de seres potencialmente submetidos a impactos negativos da tecnologia (que não se incluem entre as pragas da lavoura), os ONAs necessitam de protocolos de avaliação ajustados a suas singularidades, compreendendo os diferentes ambientes onde se estabelecem.

Toxicidade aguda, toxicidade subcrônica, alterações metabólicas que influenciem o *fitness* de determinadas espécies, suas dinâmicas populacionais e os efeitos negativos sobre o conjunto envolvido, decorrente de flutuações populacionais e inclusive de alterações na qualidade nutricional das plantas transformadas[41], incluem-se entre os itens que devem ser considerados em avaliações do risco ambiental.

O conjunto de estudos referenciados neste capítulo incluem diversos casos[42] de impactos negativos decorrentes do uso de plantas Bt, sobre seres classificados como ONAs dessa tecnologia. Algumas situações incorporam divergências de interpretação científica sobre relações entre causas e consequências. De fato, algumas polêmicas relativas ao real impacto ambiental da tecnologia sobre determinadas espécies de insetos se mantém desde a década anterior. Destacam-se os exemplos emblemáticos da borboleta Monarca[43] (*Danaus plexippus*), da larva do neuroptera *Chrysoperla carnea* e da joaninha *Adalia bipunctata*.

REV – Hilbeck, A. 2001. Implications of transgenic, insecticidal plants for insect and plant biodiversity. *Perspectives in Plant Ecology, Evolution and Systematics*, 4(1), 43-61.

Genetically modified plants are widely grown predominantly in North America and to a lesser extent in Australia, Argentina and China but their regions of production are expected to spread soon beyond these limited areas also reaching Europe where great controversy over the application of gene technology in agriculture persists. Currently, several cultivars of eight major crop plants are commercially available including canola, corn, cotton, potato, soybean, sugar beet, tobacco and tomato, but many more plants with new and combined multiple traits are close to registration. While currently agronomic traits (herbicide resistance, insect resistance) dominate, traits conferring “quality” traits (altered oil compositions, protein and starch contents) will begin to dominate within the next years. However, economically the most promising future lies in the development and marketing of crop plants expressing pharmaceutical or “nutraceuticals” (functional foods), and plants that express a number of different genes. From this it is clear that future agricultural and, ultimately, also natural ecosystems will be challenged by the large-scale introduction of entirely novel genes and gene products in new combinations at high frequencies all of which will have unknown impacts on their associated complex of non-target organisms, i.e. all organisms that are not targeted by the insecticidal protein. In times of severe global decline of biodiversity, pro-active precaution is necessary and careful consideration of the likely expected effects of transgenic plants on biodiversity of plants and insects is mandatory.

In this paper possible implications of non-target effects for insect and plant biodiversity are discussed and a case example of such non-target effects is presented. In a multiple year research project, tritrophic and bitrophic effects of transgenic corn, expressing the gene from *Bacillus thuringiensis* (Bt-corn) that codes for the high expression of an insecticidal toxin (Cry1Ab), on the

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[41] No item 2.3 da Parte 1 incluem-se artigos que exemplificam diferenças nutricionais e composicionais entre plantas Bt e convencionais.

[42] O conhecimento atual a respeito de impactos potenciais das toxinas Cry sobre ONAs permite afirmar que os estudos aqui relacionados representam somente “a ponta do iceberg”, sendo ilustrativos de algo que ocorre com outras centenas de espécies de artrópodes, nos diversos países que adotam a tecnologia Bt.

[43] No item item 4.2.1 da Parte 5 incluem-se artigos relevantes sobre essa polêmica científica.

natural enemy species, *Chrysoperla carnea* (the green lacewing), was investigated. In these laboratory trials, we found prey-mediated effects of transgenic Bt-corn causing significantly higher mortality of *C. carnea* larvae. In further laboratory trials, we confirmed that the route of exposure (fed directly or via a herbivorous prey) and the origin of the Bt (from transgenic plants or incorporated into artificial diet) strongly influenced the degree of mortality. In choice feeding trials where *C. carnea* could choose between *Spodoptera littoralis* fed transgenic Bt-corn and *S. littoralis* fed non-transgenic corn, larger instars showed a significant preference for *S. littoralis* fed non-transgenic corn while this was not the case when the choice was between Bt- and isogenic corn fed aphids. Field implications of these findings could be multifold but will be difficult to assess because they interfere in very intricate ways with complex ecosystem processes that we still know only very little about. The future challenge in pest management will be to explore how transgenic plants can be incorporated as safe and effective components of IPM systems and what gene technology can contribute to the needs of a modern sustainable agriculture that avoids or reduces adverse impacts on biodiversity? For mainly economically motivated resistance management purposes, constitutive high expression of Bt-toxins in transgenic plants is promoted seeking to kill almost 100% of all susceptible (and if possible heterozygote resistant) target pest insects. However, for pest management this is usually not necessary. Control at or below an established economic injury level is sufficient for most pests and cropping systems. It is proposed that partially or moderately resistant plants expressing quantitative rather than single gene traits and affecting the target pest sub-lethally may provide a more meaningful contribution of agricultural biotechnology to modern sustainable agriculture. Some examples of such plants produced through conventional breeding are presented. Non-target effects may be less severe allowing for better incorporation of these plants into IPM or biological control programs using multiple control strategies, thereby, also reducing selection pressure for pest resistance development.

http://www.sciencedirect.com/science/article/pii/S1433831904700396

REV – Marvier, M.; McCreedy, C.; Regetz, J.; Kareiva, P. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. *Science*, 316(5830): 1475-1477.

Although scores of experiments have examined the ecological consequences of transgenic Bacillus thuringiensis (Bt) crops, debates continue regarding the nontarget impacts of this technology. Quantitative reviews of existing studies are crucial for better gauging risks and improving future risk assessments. To encourage evidence-based risk analyses, we constructed a searchable database for nontarget effects of Bt crops. A meta-analysis of 42 field experiments indicates that nontarget invertebrates are generally more abundant in Bt cotton and Bt maize fields than in nontransgenic fields managed with insecticides. However, in comparison with insecticide-free control fields, certain nontarget taxa are less abundant in Bt fields.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/metamarvier.pdf

1.2.1 Efeitos negativos em ONAs que se alimentam diretamente de material vegetal Bt

Um dos maiores desafios impostos à avaliação de impactos das tecnologias Bt sobre organismos estabelecidos nos agroecossistemas por elas afetados reside na elaboração de metodologias de pesquisa que compreendam adequadamente o conjunto de efeitos potenciais relacionados à dispersão massiva das toxinas.

Se de um lado existem sinais de toxicidade aguda, facilmente detectáveis, de outro lado e mais perniciosos em termos de longo prazo estão os efeitos crônicos de subdosagens.

Os artigos a seguir apontam – com base em bioensaios e experimentos de campo – impactos negativos observados em ONAs (insetos, artrópodes aquáticos, organismos do solo) alimentados com material vegetal de origem transgênica Bt (inclusive pólen).

COM – Losey, J.; Rayor, L.; Carter, M. 1999. Transgenic pollen harms monarch larvae. *Nature*, 399 (6733): 214.

Sem resumo.

Artigo completo disponível em http://www.nature.com/scitable/content/transgenic-pollen-harms-monarch-larvae-97961

Jesse, L.; Obrycki, J. 2000. Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia*, 125:241–248. doi: 10.1007/s004420000502.

We present the first evidence that transgenic Bacillus thuringiensis (Bt) corn pollen naturally deposited on Asclepias syriaca; common milkweed, in a corn field causes significant mortality of Danaus plexippus L. (Lepidoptera: Danaidae) larvae. Larvae feeding for 48 h on A. syriaca plants naturally dusted with pollen from Bt corn plants suffered significantly higher rates of mortality at 48 h (20±3%) compared to larvae feeding on leaves with no pollen (3±3%), or feeding on leaves with non-Bt pollen (0%). Mortality at 120 h of D. plexippus larvae exposed to 135 pollen grains/cm(2) of transgenic pollen for 48 h ranged from 37 to 70%. We found no sub-lethal effects on D. plexippus adults reared from larvae that survived a 48-h exposure to three concentrations of Bt pollen. Based on our quantification of the wind dispersal of this pollen beyond the edges of agricultural fields, we predict that the effects of transgenic pollen on D. plexippus may be observed at least 10 m from transgenic field borders. However, the highest larval mortality will likely occur on A. syriaca plants in corn fields or within 3 m of the edge of a transgenic corn field. We conclude that the ecological effects of transgenic insecticidal crops need to be evaluated more fully before they are planted over extensive areas.

http://www.ncbi.nlm.nih.gov/pubmed/24595835

Hellmich, R.; Siegfried, B.; Sears, M.; Stanley-Horn, D.; Daniels, M.; Mattila, H.; Spencer, T.; Bidne, K.; Lewis, L. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis*- purified proteins and pollen. *PNAS*, 98 (21) 11925-11930.

Laboratory tests were conducted to establish the relative toxicity of *Bacillus thuringiensis* (*Bt*) toxins and pollen from *Bt* corn to monarch larvae. Toxins tested included Cry1Ab, Cry1Ac, Cry9C, and Cry1F. Three methods were used: (*i*) purified toxins incorporated into artificial diet, (*ii*) pollen collected from *Bt* corn hybrids applied directly to milkweed leaf discs, and (*iii*) *Bt* pollen contaminated with corn tassel material applied directly to milkweed leaf discs. Bioassays of purified *Bt* toxins indicate that Cry9C and Cry1F proteins are relatively nontoxic to monarch first instars, whereas first instars are sensitive to Cry1Ab and Cry1Ac proteins. Older instars were 12 to 23 times less susceptible to Cry1Ab toxin compared with first instars. Pollen bioassays suggest that pollen contaminants, an artifact of pollen processing, can dramatically influence larval survival and weight gains and produce spurious results. The only transgenic corn pollen that consistently affected monarch larvae was from Cry1Ab event 176 hybrids, currently <2% corn planted and for which re-registration has not been applied. Results from the other types of *Bt* corn suggest that pollen from the Cry1Ab (events Bt11 and Mon810) and Cry1F, and experimental Cry9C hybrids, will have no acute effects on monarch butterfly larvae in field settings.

Artigo completo disponível em http://www.pnas.org/content/98/21/11925.full.pdf+html

Zangerl, A.; McKenna, D.; Wraight, C.; Carroll, M.; Ficarello, P.; Warner, R.; Berenbaum, M. 2001. Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. *PNAS*, 98: 11908-11912.

The widespread planting of corn genetically modified to produce *Bacillus thuringiensis* endotoxin has led to speculation that pollen from these fields might adversely affect nearby nontarget lepidopterans. A previous study of *Bt* corn engineered with Monsanto event 810 failed to detect an effect of pollen exposure on the black swallowtail, *Papilio polyxenes*, in either the field or the laboratory. Here, we report results of a field study investigating the impact of exposure to pollen from a *Bt* corn hybrid containing Novartis event 176 on two species of Lepidoptera, black swallowtails and monarch butterflies, *Danaus plexippus*. Nearly half of the 600 monarch larvae died within the first 24 h; this and subsequent mortality was not associated with proximity to *Bt* corn and may have been due in part to predation. Survivorship of black swallowtails was much higher than that of the monarchs and was also independent of proximity to the transgenic corn. However, despite five rainfall events that removed much of the pollen from the leaves of their host plants during the experiment, we observed a significant reduction in growth rates of black swallowtail larvae that was likely caused by pollen exposure. These results suggest that *Bt* corn incorporating event 176 can have adverse sublethal effects on black swallowtails in the field and underscore the importance of event selection in reducing environmental impacts of transgenic plants.

Artigo completo disponível em http://www.pnas.org/content/98/21/11908.full

Zwahlen, C.; Hilbeck, A.; Howald, R.; Nentwig, W. 2003. Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*. Molecular Ecology, 12 (8):1077 1086.

A 200-day study was carried out to investigate the impact of transgenic Bacillus thuringiensis (Bt) corn on immature and adult Lumbricus terrestris in the field and in the laboratory. Another objective of this study was to develop test methods that could be used for standard testing of the impact of transgenic plants on different earthworm species in the field and in the laboratory. For this purpose two different experiments were involved, a laboratory experiment with adult L. terrestris and a field experiment with immature L. terrestris. No lethal effects of transgenic Bt corn on immature and adult earthworms were observed. Immature L. terrestris in the field had a very similar growth pattern when fed either (Bt+) or (Bt-) corn litter. No significant differences in relative weights of (Bt+) and (Bt-) corn-fed adult L. terrestris were observed during the first 160 days of the laboratory trial, but after 200 days adult L. terrestris had a significant weight loss of 18% of their initial weight when fed (Bt+) corn litter compared to a weight gain of 4% of the initial weight of (Bt-) corn-fed earthworms. Further studies are necessary to see whether or not this difference in relative weight was due to the Bt toxin or other factors discussed in the study. Degradation of Cry1Ab toxin in corn residues was significantly slower in the field than at 10 degrees C in the laboratory. Enzyme-linked immunosorbent assay results indicated that earthworms in both experiments were exposed to the Bt toxin throughout the whole experimental time.

http://www.ncbi.nlm.nih.gov/pubmed/12753225

Dively, G.; Rose, R.; Sears, M.; Hellmich, R.; Stanley-Horn, D.; Calvin, D.; Russo, J.; Anderson, P. 2004. Effects on monarch butterfly larvae (*Lepidoptera: Danaidae*) after continuous exposure to Cry1Ab expressing corn during anthesis. *Environmental Entomology*, 33: 1116-1125.

Effects on monarch butterfly, *Danaus plexippus* L., after continuous exposure of larvae to natural deposits of *Bacillus thuringiensis* (Bt) and non-Bt pollen on milkweed, were measured in five studies. First instars were exposed at 3Ð4 and 6Ð7 d after initial anthesis, either directly on milkweed plants in commercial corn-fields or in the laboratory on leaves collected from milkweeds in corn plots. Pollen exposure levels ranging from 122 to 188 grains/cm2/d were similar to within-field levels that monarch butterfly populations might experience in the general population of cornÞelds. Results indicate that 23.7% fewer larvae exposed to these levels of Bt pollen during anthesis reached the adult stage.A risk assessment procedure used previously was updated with a simulation model estimating the proportion of second-generation monarch butterflies affected. When considered over the entire range of the Corn Belt, which represents only 50% of the breeding population, the risk to monarch butterßy larvae associated with long-term exposure to Bt corn pollen is 0.6% additional mortality. Exposure also prolonged the developmental time of larvae by 1.8 d and reduced the weights of both pupae and adults by 5.5%. The sex ratio and wing length of adults were unaffected. The ecological significance of these sublethal effects is discussed relative to generation mortality and adult performance.

Artigo completo disponível em http://www.biosicherheit.de/pdf/dokumente/bt-monarch-maryland.pdf

Yoichi, S.; Mami, T. 2005. Effects of transgenic Bt corn pollen on a non-target lycaenid butterfly, *Pseudozizeeria maha. Applied Entomology and Zoology*, 40 (1): 151–159.

To evaluate the effect of pollen released from transgenic insecticidal corn on non-target lepidopteran insects, corn pollen deposition density on the leaves of sunflower and black nightshade was measured near a cornfield. At 12 d from the start of anthesis, the highest cumulative pollen density on leaves was approximately 160 grains per cm^2 at 1m from the edge of the cornfield, falling to 20 grains at 5 m and less than 10 grains at 10m. The pollen density calculated using a mathematical model in a previous study evidently had overestimated values. To evaluate precisely the effect of corn pollen expressing Bacillus thuringiensis (Bt) endotoxin (Cry1Ab) on the survival of lepidopteran larvae, we improved the bioassay methods using the pale grass blue, Pseudozizeeria maha, the leaf disc of the wood sorrel, Oxalis corniculata, and transgenic Bt corn (Event-176). When the surface of the leaf was pretreated with a small amount of 80% acetone solution, the preselected pollen dose was successfully applied onto the leaf disc. Larval survival of P. maha was significantly affected at pollen density of more than 20 grains per cm^2 on the leaf disc. It is unlikely that pollens from Bt corn expressing Cry1Ab have wide-scaled deleterious effects on non-target P. maha near cornfields, because of low pollen deposition dose on the leaves.

http://ci.nii.ac.jp/naid/10014474578/en

Lang, A.; Vojtech, E. 2006. The effects of pollen consumption of transgenic Bt maize on the common swallowtail, *Papilio machaon* L. (*Lepidoptera, Papilionidae*). *Basic and Applied Ecology*, 7: 296-306.

Effects of exposure to maize pollen of event Bt176 (cultivar "Navares") on the larvae of the European common swallowtail (*Papilio machaon* L.) were studied in the laboratory. First instar larvae were exposed to different pollen densities applied to leaf disks of *Pastinaca sativa* L. for 48 h. Pollen densities applied in this study were in the range recorded from the field. Larvae which were exposed to higher Bt maize pollen densities consumed more pollen and had a lower survival rate. The LD_{50}

with regard to larvae surviving to adulthood was 13.72 pollen grains consumed by first-instar larva. Uptake of Bt maize pollen led to a reduced plant consumption, to a lower body weight, and to a longer development time of larvae. Effects on pupal weight and duration of the pupal period were present but less pronounced and smaller than effects on larvae. Larvae having consumed Bt-maize pollen as first instars had a lower body weight as adult females and smaller forewings as adult males. We conclude that possible effects of Bt maize on European butterflies and moths must be evaluated more rigorously before Bt maize should be cultivated over large areas.

http://www.sciencedirect.com/science/article/pii/S1439179105001246

Rosi-Marshall, E.; Tank, J.; Royer, T.; Whiles, M.; Evans-White, M.; Chambers, C.; Griffiths, N.; Pokelsek, J.; Stephen, M. 2007. Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *PNAS*, 104(41): 16204-16208.

Corn (*Zea mays* L.) that has been genetically engineered to produce the Cry1Ab protein (Bt corn) is resistant to lepidopteran pests. Bt corn is widely planted in the midwestern United States, often adjacent to headwater streams. We show that corn byproducts, such as pollen and detritus, enter headwater streams and are subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials showed that consumption of Bt corn byproducts reduced growth and increased mortality of nontarget stream insects. Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystem-scale consequences.

Artigo completo disponível em http://www.pnas.org/content/104/41/16204.full

Bøhn, T.; Primicerio, R.; Hessen, D.; Traavik, T. 2008. Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. *Archives of Environmental Contamination and Toxicology*, DOI 10.1007/s00244-008-9150-5.

Genetically modified (GM) maize expressing the Bt-toxin Cry1Ab (Bt-maize) was tested for effects on survival, growth, and reproduction of the water flea Daphnia magna, a crustacean arthropod commonly used as a model organism in ecotoxicological studies. In three repeated experiments, D. magna were fed 100% ground maize in suspension, using either GM or isogenic unmodified (UM) maize. D. magna fed GM-maize showed a significantly reduced fitness performance: The mortality was higher, a lower proportion of females reached sexual maturation, and the overall egg production was lower compared to D. magna fed UM isogenic maize. We conclude that the tested variety of Bt-maize and its UM counterpart do not have the same quality as food sources for this widely used model organism. The combination of a reduced fitness performance combined with earlier onset of reproduction of D. magna fed Bt-maize indicates a toxic effect rather than a lower nutritional value of the GM-maize.

http://www.ncbi.nlm.nih.gov/pubmed/18347840

Mason, C.; Sheldon, J.; Pesek, J.; Bacon, H.; Gallusser, R.; Radke, G.; Slabaugh, B. 2008. Assessment of *Chrysoperla plorabunda* longevity, fecundity, and egg viability when adults are fed transgenic Bt corn pollen. *Journal of Agricultural and Urban Entomology*, 25(4): 265–278.

The widespread planting of transgenic corn containing Bacillus thuringiensis (Bt) Cry endotoxin in its tissues for insect pest control raises the potential for influence on many non-target species including pollenfeeding species of Chrysopidae. This study was conducted to assess fitness parameters associated with longevity, fecundity, and egg viability of adult Chrysoperla plorabunda

(Fitch) (Neuroptera: Chrysopidae) when fed Bt corn pollen. Bt products tested with their respective non-Bt near-isolines were Event 176 (Cry1Ab), MON810 (Cry1Ab), and TC1507 (Cry1F). Females fed pollen from Event 176 lived significantly longer than those fed pollen from its non-Bt near-isoline. Males fed pollen from TC1507 showed a trend for living longer than males fed its non-Bt near-isoline pollen, but there was no difference for females regarding this event. The mean number of eggs produced per female per day was significantly less for those fed MON810 pollen compared with females fed pollen from the non-Bt near-isoline. Total egg production was significantly less for females fed MON810 pollen vs. females fed pollen from its non-Bt near-isoline.

http://www.bioone.org/doi/abs/10.3954/1523-5475-25.4.265

Höss, S.; Arndt, M.; Baumgarte, S.; Tebbe, C.; Nguyen, H.; Jehle, J. 2008. Effects of transgenic corn and Cry1Ab protein on the nematode, *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*, 70 (2): 334-340.

The effects of the insecticidal Cry1Ab protein from Bacillus thuringiensis (Bt) on the nematode, Caenorhabditis elegans, were studied with soil from experimental fields cultivated with transgenic Bt corn (MON810) and with trypsinized Cry1Ab protein expressed in Escherichia coli. The content of Cry1Ab protein was above the detection limit of an ELISA test in only half of the soil samples obtained from transgenic plots, ranging from 0.19 to 1.31 ng g_1 dry weight. In a laboratory bioassay, C. elegans was exposed to rhizosphere and bulk soil from fields with isogenic or transgenic corn or to solutions of Cry1Ab protein (0, 24, 41, 63, 118, and 200 mgl_1) over a period of 96 h, with growth and reproduction serving as the test parameters. Nematode reproduction and growth were significantly reduced in rhizosphere and bulk soil of Bt corn compared with soil from isogenic corn and were significantly correlated with concentrations of the Cry1Ab protein in the soil samples. Moreover, the toxicity of pure Cry1Ab protein to the reproduction and growth of C. elegans was concentration-dependent. As significant inhibition occurred at relatively high concentrations of the Cry1Ab protein (41 mgl_1), the effects of the soil samples from Bt corn could not be assigned directly to the toxicity of the Cry1Ab protein. The results demonstrate that bioassays with the nematode, C. elegans, provide a promising tool for monitoring the potential effects of Bt toxins in aqueous médium and soils.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/caenorhabditishoess.pdf

Schmidt, J.; Braun, C.; Whitehouse, L.; Hilbeck, A. 2009. Effects of activated Bt transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird *Adalia bipunctata* in Laboratory Ecotoxicity Testing. *Arch Environ Contam Toxicol*, 56:221–228.

Insect-active Bacillus thuringiensis (Bt) proteins are expressed by several transgenic crop plants to control certain pests, but nontarget organisms such as ladybirds also can be exposed to these proteins in the field. We developed an improved ecotoxicity testing protocol and conducted feeding trials in a laboratory setting to test for possible adverse effects of different concentrations of microbially produced trypsin-activated Cry1Ab and Cry3Bb toxins on the coccinellid Adalia bipunctata. Larval/pupal mortality, development time, and overall body mass accumulation were recorded. Even at the lowest concentration (5 microg/ml), A. bipunctata larvae fed with the lepidopteran-active Cry1Ab toxin exhibited significantly higher mortality than the control group. In experiments with the coleopteran-active Cry3Bb, only a concentration of 25 microg/ml resulted in a marginally significantly higher mortality compared to the control. Both experiments revealed a slight decline in mortality at the highest concentration of 50 microg/ml, though this was statistically significant only in the Cry1Ab treatment. No differences were detected for development time and body mass of newly emerged adults. Dilutions of the expression vector pBD10--used as a control to exclude effects of the toxin production method--at concentrations between 10 and 100 microg/ml revealed no significant effects on either of the studied parameters. This suggests that the increased mortality of larvae in the toxin feeding trials was caused directly by the activated Bt toxins and raises questions regarding their commonly postulated specificity and their mode of action in A. bipunctata. Implications of the reported results for ladybird populations and their biological pest control functions in transgenic crop ecosystems are discussed.

Artigo Completo em http://stopogm.net/sites/stopogm.net/files/cry3Bbschmidt.pdf

REV - Lang, A.; Otto, M. 2010. A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target *Lepidoptera*. *Entomologia Experimentalis et Applicata*, 135: 121–134, 2010.

One of the major applications of transgenic crops in agriculture are the so-called Bacillus thuringiensis Berliner (Bt) plants, in particular Bt maizes, which produce insecticidal Cry proteins that target specific orders, such as the Lepidoptera or Coleoptera. We reviewed publications that reported on the direct toxic effects of Bt-maize and/or Cry proteins of current Bt-maize events on larvae of non-target butterflies and moths (Lepidoptera). In total, 20 peer-reviewed publications were identified, of which 16 papers contributed laboratory-based data and seven field-based data. An adverse effect on caterpillars was recorded in 52% of all laboratory-based and in 21% of all field-based observations. The variables most often studied and having the highest occurrence of effects were larval survival, body mass, and developmental time. Parameters of the adult stage were under-represented in the studies. Overall, 11 lepidopteran species were tested. The majority of the studies originated from the USA, with the Monarch butterfly being the most studied, whereas other species and other parts of the world were widely neglected. Laboratory experiments were often run under unrealistic conditions from an ecological point of view. Although the papers we reviewed indicated a potential hazard for Lepidoptera that are exposed to and feed on lepidopteran-specific Bt-maize pollen, a general conclusion on the level of risk for butterflies and moths cannot as yet be drawn. A comprehensive risk characterization would require thorough hazard identification, exposure assessment, and impact assessment. However, our review showed that even the basic level of hazard characterization is as yet incomplete. Reasons for this are the still-limited numbers of publications and concurrent lack of knowledge, the restriction of data to only a few species, the over-representation of North American species, and the identified limitations of both laboratory and field experiments. The findings of this review suggest that more realistic, ecologically meaningful, and detailed experiments and analyses are crucial to improve the present assessment of Bt-maize cultivation effects on Lepidoptera.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1570-7458.2010.00981.x/pdf

Jensen, P.; Dively, G.; Swan, C.; Lamp, W. 2010. Exposure and nontarget effects of transgenic Bt corn debris in streams. *Environmental Entomology*, 39 (2) 707-714.

Corn (Zea mays L.) transformed with a gene from the bacterium Bacillus thuringiensis (Bt) comprises 49% of all corn in the United States. The input of senesced corn tissue expressing the Bt gene may impact stream-inhabiting invertebrates that process plant debris, especially trichopteran species related to the target group of lepidopteran pests. Our goal was to assess risk associated with transgenic corn debris entering streams. First, we show the input of corn tissue after harvest was extended over months in a stream. Second, using laboratory bioassays based on European corn borer [Ostrinia nubilalis (Hübner)], we found no bioactivity of Cry1Ab protein in senesced corn tissue after 2 wk of exposure to terrestrial or aquatic environments. Third, we show that Bt near-isolines modify growth and survivorship of some species of invertebrates. Of the four nontarget invertebrate species fed Bt near-isolines, growth of two closely related trichopterans was not negatively affected, whereas a tipulid crane fly exhibited reduced growth rates, and an isopod exhibited reduced growth and survivorship on the Cry1Ab near-isoline but not on the stacked Cry1Ab + Cry3Bb1 near-isoline. Because of lack of evidence of bioactivity of Bt after 2 wk and because of lack of nontarget effects on the stacked near-isoline, we suggest that tissue-mediated differences, and not the presence of the Cry1Ab protein, caused the different responses among the species. Overall, our results provide evidence that adverse effects to aquatic nontarget shredders involve complex interactions arising from plant genetics and environment that cannot be ascribed to the presence of Cry1Ab proteins.

Artigo completo disponível em http://ee.oxfordjournals.org/content/ee/39/2/707.full.pdf

Bøhn, T.; Traavik, T.; Primicerio, R. 2010. Demographic responses of *Daphnia magna* fed transgenic Bt-maize. *Ecotoxicology*, 19(2): 419-430.

The food/feed quality of a variety of genetically modified (GM) maize expressing Cry1Ab Bt-toxin was tested over the life-cycle of *Daphnia magna*, an arthropod commonly used as model organism in ecotoxicological studies. Demographic responses were compared between animals fed GM or unmodified (UM) near isogenic maize, with and without the addition of predator smell. Age-specific data on survival and birth rates were integrated and analysed using life tables and Leslie matrices. Survival, fecundity and population growth rate (PGR) data generally disfavoured transgenic Bt-maize as feed for *D. magna* compared to animals fed the unmodified (UM) near isogenic line of maize. Decomposition of age-specific effects revealed that the most important contributions to a reduced PGR in the GM-fed group came from both fecundity and survival differences early in life. We conclude that juvenile and young adult stages are the most sensitive experimental units and should be prioritized in future research. These stages are often omitted in toxicological/ecotoxicological studies and in feeding trials.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2811247/

Hilbeck, A.; McMillan, J.; Meier, M.; Humbel, A.; Schlaepfer-Miller, J.; Trtikova, M. 2012. A controversy re-visited: Is the coccinellid *Adalia bipunctata* adversely affected by Bt toxins? *Environmental Sciences Europe*, 24(10).

Background: In 2008/2009, Schmidt and colleagues published a study reporting lethal effects of the microbial Bt toxins Cry1Ab and Cry3Bb on the coccinellid biological control organisms *Adalia bipunctata*. Based on this study, in concert with over 30 other publications, Mon810 cultivation was banned in Germany in 2009. This triggered two commentaries and one experimental study all published in the journal '*Transgenic Research*' that question the scientific basis of the German ban or claim to disprove the adverse effects of the Bt toxins on *A. bipunctata* reported by Schmidt and colleagues, respectively. This study was undertaken to investigate the underlying reasons for the different outcomes and rebuts the criticism voiced by the two other commentaries.
Results: It could be demonstrated that the failure to detect an adverse effect by Alvarez-Alfageme and colleagues is based on the use of a significantly different testing protocol. While Schmidt and colleagues exposed and fed larvae of *A. bipunctata* continuously, Alvarez-Alfageme and colleagues applied an exposure/recovery protocol. When this exposure/recovery protocol was applied to a highly sensitive target insect, *Ostrinia nubilalis*, the lethal effect was either significantly reduced or disappeared altogether. When repeating the feeding experiments with the Bt toxin Cry1Ab using a combined protocol of both previous studies, again, a lethal effect on *A. bipunctata* larvae was observed. ELISA tests with Bt-toxin fed larvae and pupae confirmed ingestion of the toxin.
Conclusions: The new data corroborates earlier findings that Cry1Ab toxin increases mortality in *A. bipunctata* larvae. It was also shown that the different applied testing protocols explained the contrasting results.

Artigo completo disponível em http://www.enveurope.com/content/24/1/10

MOD –Holst, N.; Lang, A.; Lövei, G.; Otto, M. 2013. Increased mortality is predicted of *Inachis io* larvae caused by Bt-maize pollen in European farmland. *Ecological Modelling*, 250: 126133.

A potential environmental risk of the field cultivation of insect-resistant (Bt-toxin expressing) transgenic maize (*Zea mays*) is the consumption of Bt-containing pollen by herbivorous larvae of butterflies (Lepidoptera). Maize is wind-pollinated, and at flowering time large amounts of pollen can be deposited on various plants growing in the landscape, leading to inadvertent ingestion of toxic pollen with plant biomass consumed by these butterfly larvae. To examine the possible effect of this coincidence, we focused our study on the protected butterfly *Inachis io* and two regions of Europe. Using climatic records, maize and butterfly phenology data, we built a simulation model of the butterfly's annual life cycle, overlaid with the phenology of maize pollen deposition on the leaves of the food plant *Urtica dioica*, and linked these with the dose–response curve of *I. io* larvae to Bt-maize pollen (event MON810). The simulations indicated that in Northern Europe, where *I. io* is univoltine, Bt-maize pollen would not be present on the food plant at the same time as the *I. io* larvae. However, in Central and Southern Europe, where *I. io* is bivoltine, Bt-maize pollen and the second generation *I. io* larvae would coincide, and an increased mortality of the larvae was predicted. This prediction differs from earlier studies which predicted negligible effect of field-grown Bt-maize on *I. io* larvae. Our model is an improvement over previous efforts since it is based on more detailed, empirical data, includes more biological detail, and provides explicit estimation of all model parameters. The model is open-source software and is available for re-use and for modelling the effects on other species or regions.

http://www.sciencedirect.com/science/article/pii/S0304380012005315

Linn, M.; Moore, P. 2014. The effects of Bt corn on rusty crayfish (*Orconectes rusticus*) growth and survival. *Arch Environ Contam Toxicol*, 67(3):436-43. doi: 10.1007/s00244-014-0061-3.

Bt crops are one of the most commonly used genetically modified crops worldwide. Bt crops contain a gene that is derived from the bacteria Bacillus thuringiensis, which produces the Cry1Ab toxin. Bt corn that contains the Cry1Ab toxin is used throughout the Midwest United States to control crop pests such as the European corn borer (Ostrinia nubilalis). Headwater streams in regions known for intensive agriculture receive Bt corn detritus after the fall harvest, which is then consumed by a diverse community of stream invertebrates. The rusty crayfish (Orconectes rusticus) is a common invertebrate detritivore in these headwater streams. Both isogenic and Bt corn were grown under the controlled environmental conditions of a greenhouse and, after senescence, were tested for nutritional equality. Rusty crayfish were exposed to one of several detrital treatments composed of Bt corn, Bt corn plus American sycamore (Platanus occidentalis), isogenic corn alone, isogenic corn plus P. occidentalis, or P. occidentalis alone for 8 weeks. Both strains of corn were grown under the controlled environmental conditions in a greenhouse and were tested for nutritional equality after senescence. Crayfish were housed in live streams with a water temperature of 12.8 °C and a 12:12 h light-to-dark photoperiod. Survival and growth of animals within each experimental treatment were monitored each week. After 8 weeks of exposure, there was no statistically significant difference in growth between crayfish in Bt and isogenic treatments. However, survivorship was 31 % lower in the Bt treatment compared with the isogenic treatment. These results suggest that the Bt corn and isogenic corn were of equivalent nutritional value but that Bt corn does have a toxic effect on rusty crayfish during long-term exposure.

http://www.ncbi.nlm.nih.gov/pubmed/25001246

Dificilmente percebidos em laboratórios, impactos subcrônicos podem se concretizar por intermédio de alterações comportamentais envolvendo os organismos afetados e suas relações com o meio ambiente.

Nesse contexto, entre os poucos estudos realizados nos campos da sociobiologia e da etiologia, destacam-se avaliações de danos sobre populações de abelhas domésticas. Os resultados apontam alterações comportamentais que resultam em danos e desequilíbrios de ordem populacional.

Prasifka, P.; Hellmich, R.; Prasifka, J.; Lewis, L. 2007. Effects of Cry1Ab-expressing corn anthers on the movement of monarch butterfly larvae. *Environmental Entomology*, 36 (1): 228-33.

Decreased larval feeding and weight of the monarch butterfly, Danaus plexippus L., have been detected after 4 d of exposure in the laboratory to a high density of Bacillus thuringiensis (Bt)-expressing anthers. One hypothesis is that larvae exposed to Bt anthers exhibit increased wandering, resulting in less feeding and lower weight gain. To test this hypothesis, 2-d-old monarch butterfly larvae exposed to milkweed leaf disks with no anthers, anthers that express Bt (Cry1Ab, event MON810), or other non-Bt anthers were observed using a video-tracking system. As had been shown in previous studies, larvae exposed to Bt anthers fed less and gained less weight than larvae exposed to non-Bt or no anthers, yet there was no evidence of feeding on anthers. Total distance moved, maximum displacement from release point, percentage of time spent moving or near anthers, or mean turn angle did not differ across treatments. However, larvae exposed to Bt anthers spent more time off milkweed leaf disks than those exposed to no anthers and were more likely to move off the leaf than larvae exposed to non-Bt anthers. Results suggest that larvae exposed to Bt anthers behave differently and that ingestion may not be the only way Bt can affect nontarget insects like the monarch butterfly.

Artigo completo disponível em http://ee.oxfordjournals.org/content/ee/36/1/228.full.pdf

Ramirez-Romero, R.; Desneux, N.; Decourtye, A.; Chaffiol, A.; Pham-Delègue, M. 2008. Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (*Hymenoptera*, *Apidae*)? *Ecotoxicology and Environmental Safety*, 70 (3): 327 333.

Genetically modified Bt crops are increasingly used worldwide but side effects and especially sublethal effects on beneficial insects remain poorly studied. Honey bees are beneficial insects for natural and cultivated ecosystems through pollination. The goal of the present study was to assess potential effects of two concentrations of Cry1Ab protein (3 and 5000 ppb) on young adult honey bees. Following a complementary bioassay, our experiments evaluated effects of the Cry1Ab on three major life traits of young adult honey bees: (a) survival of honey bees during sub-chronic exposure to Cry1Ab, (b) feeding behaviour, and (c) learning performance at the time that honey bees become foragers. The latter effect was tested using the proboscis extension reflex (PER) procedure. The same effects were also tested using a chemical pesticide, imidacloprid, as positive reference. The tested concentrations of Cry1Ab protein did not cause lethal effects on honey bees. However, honey bee feeding behaviour was affected when exposed to the highest concentration of Cry1Ab protein, with honey bees taking longer to imbibe the contaminated syrup. Moreover, honey bees exposed to 5000 ppb of Cry1Ab had disturbed learning performances. Honey bees continued to respond to a conditioned odour even in the absence of a food reward. Our results show that transgenic crops expressing Cry1Ab protein at 5000 ppb may affect food consumption or learning processes and thereby may impact honey bee foraging efficiency. The implications of these results are discussed in terms of risks of transgenic Bt crops for honey bees.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/hymenopteraramirez.pdf

Han, P.; Niu, C-Y.; Lei, C-L.; Cui, J-J.; Desneux, N. 2010. Quantification of toxins in a Cry1Ac + CpTI cotton cultivar and its potential effects on the honey bee *Apis mellifera* L. *Ecotoxicology*, 19(8):1452-9.

Transgenic Cry1Ac + CpTI cotton (CCRI41) is increasingly planted throughout China. However, negative effects of this cultivar on the honey bee Apis mellifera L., the most important pollinator for cultivated ecosystem, remained poorly investigated. The objective of our study was to evaluate the potential side effects of transgenic Cry1Ac + CpTI pollen from cotton on young adult honey bees A. mellifera L. Two points emphasized the significance of our study: (1) A higher expression level of insecticidal protein Cry1Ac in pollen tissues was detected (when compared with previous reports). In particular, Cry1Ac protein was detected at 300 ± 4.52 ng g(-1) [part per billion (ppb)] in pollen collected in July, (2) Effects on chronic mortality and feeding behaviour in honey bees were evaluated using a no-choice dietary feeding protocol with treated pollen, which guarantee the highest exposure level to bees potentially occurring in natural conditions (worst case scenario). Tests were also conducted using imidacloprid-treated pollen at a concentration of 48 ppb as positive control for sublethal effect on feeding behaviour. Our results suggested that Cry1Ac + CpTI pollen carried no lethal risk for honey bees. However, during a 7-day oral exposure to the various treatments (transgenic, imidacloprid-treated and control), honey bee feeding behaviour was disturbed and bees consumed significantly less CCRI41 cotton pollen than in the control group in which bees were exposed to conventional cotton pollen. It may indicate an antifeedant effect of CCRI41 pollen on honey bees and thus bees may be at risk because of large areas are planted with transgenic Bt cotton in China. This is the first report suggesting a potential sublethal effect of CCRI41 cotton pollen on honey bees. The implications of the results are discussed in terms of risk assessment for bees as well as for directions of future work involving risk assessment of CCRI41 cotton.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2995320/

1.2.2 Efeitos negativos em ONAs que consomem indiretamente material vegetal Bt (por meio das cadeias tróficas)

Estudos mostram que, além de impactos potenciais relacionados ao consumo direto de material vegetal Bt, existem danos relativos ao consumo indireto, envolvendo o acúmulo das toxinas ao longo de cadeias tróficas. Isso se deve ao fato de que as toxinas Bt permanecem biologicamente ativas (são, então, acessadas por predadores não fitófagos). Observa-se também que alterações da qualidade nutricional das presas e dos hospedeiros de parasitoides (decorrentes de alimentação baseada em material Bt, por esses organismos) poderão gerar impactos negativos sobre espécies localizadas em escalas superiores das redes tróficas e ecológicas.

Hilbeck, A.; Baumgartner, M.; Fried, P.; Bigler, F. 1998. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla cornea* (*Neuroptera: Chrysopidae*). *Environmental Entomology*, 276: 480-487.

Laboratory feeding experiments using transgenic *Bacillus thuringiensis* variety *kurstaki* (Berliner) corn plants have been carried out to study the effects of *B. thuringiensis*-fed herbivores (i.e., prey), on the predator *Chrysoperla carnea* Stephens. Host plants were a transgenic *B. thuringiensis*-expressing (CrylAb) corn hybrid and the corresponding untransformed, *B. thuringiensis*-free corn hybrid. Two different prey species were used in the experiments, the European corn borer, *Ostrinia nubilalis* (Hübner) (lepidopterous target pest), and *Spodoptera littoralis* (Boisduval) (lepidopterous nontarget pest for *B. thuringiensis*). The objectives were to quantify the effects of *B. thuringiensis*-fed prey on chrysopid immature development and to determine whether observed effects were caused by sick, suboptimal prey (indirect effects) or associated with *B. thuringiensis*-related causes (direct effects). Mean total immature mortality for chrysopid larvae raised on *B. thuringiensis*-fed prey was 62% compared with 37% when raised on *B. thuringiensis*-free prey. There was no significant difference in mortality between chrysopid larvae reared on *B. thuringiensis*-fed *O. nubilalis* or *B. thuringiensis*-fed *S. littoralis*. Similarly, no significant difference in mortality was detected when chrysopid larvae were raised on *B. thuringiensis*-free *O. nubilalis* or *B. thuringiensis*-free *S. littoralis*. Development time of chrysopid larvae was prolonged when *B. thuringiensis*-fed *O. nubilalis* was given to the predators but not for *B. thuringiensis*-fed *S. littoralis*. Although some unnoticed adverse effects in *S. littoralis* may have occurred because of the *B. thuringiensis* corn, our results suggest that the reduced fitness of chrysopid larvae was associated with *B. thuringiensis*. The prolonged development time of chrysopid larvae raised on *B. thuringiensis*-fed *O. nubilalis* was probably because of a combined effect of *B. thuringiensis* exposure and nutritional deficiency caused by sick prey.

http://www.researchgate.net/publication/233639938_Effects_of_transgenic_Bacillus_thuringiensis_corn-fed_prey_on_mortality_and_development_time_of_immature_Chrysoperla_cornea_(Neuroptera_Chrysopidae)

Hilbeck, A.; Moar, W.; Pusztai-Carey, M.; Filippini, A.; Bigler, F. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea. Entomologia Experimentalis et Applicata*, 91: 305–316.

Laboratory feeding experiments were carried out to study prey-mediated effects of artificial diet containing Bacillus thuringiensis proteins on immature Chrysoperla carnea. Activated Cry1Ab toxin and the protoxins of Cry1Ab and Cry2A were mixed into standard meridic diet for Spodoptera littoralis (Boisduval) larvae at the following concentrations; for Cry1Ab toxin, 25, 50, 100gg–1 diet were used; for Cry1Ab protoxin, the concentration was doubled (50gg–1 diet, 100gg–1 diet and 200gg–1 diet) to give relative comparable levels of toxin concentration. Cry2A protoxin was incorporated into the meridic diet at one concentration only (100gg–1 diet). For the untreated control, the equivalent amount of double distilled water was added to the meridic diet. Individual C.carnea larvae were raised on S.littoralis larvae fed with one of the respective treated meridic diets described above. The objectives were to quantify and compare the resulting effects on mortality and development time of C.carnea with those observed in two previous studies investigating prey-mediated effects of transgenic Cry1Ab toxin-producing corn plants and the other studying effects of Cry1Ab toxin fed directly to C.carnea larvae. Mean total immature mortality for chrysopid larvae reared on B.thuringiensis-fed prey was always significantly higher than in the control (26%). Total immature mortality of C.carnea reared on Cry1Ab toxin 100gg–1 diet-fed prey was highest (78%) and declined with decreasing toxin concentration. Cry1Ab protoxin-exposed C.carnea larvae did not exhibit a dose response. Prey-mediated total mortality of Cry1Ab protoxin-exposed chrysopid larvae was intermediate (46–62%) to Cry1Ab toxin exposed (55–78%) and Cry2A protoxin (47%) exposed C.carnea. In agreement with the previous studies, total development time of C.carnea was not consistently, significantly affected by the Bt-treatments except at the highest Cry1Ab toxin concentration. However, both highest mortality and delayed development of immature C.carnea raised on Cry1Ab toxin 100gg–1 diet – fed prey may have been confounded with an increased intoxication of S.littoralis larvae that was observed at that concentration. At all other B.thuringiensis protein concentrations S.littoralis was not lethally affected. Comparative analysis of the results of this study with those of the two previous studies revealed that in addition to prey/herbivore by B.thuringiensis interactions, also prey/herbivore by plant interactions exist that contribute to the observed toxicity of B.thuringiensis – fed S.littoralis larvae for C.carnea. These findings demonstrate that tritrophic level studies are necessary to assess the long-term compatibility of insecticidal plants with important natural enemies.

http://www.researchgate.net/publication/226586628_Prey-mediated_effects_of_Cry1Ab_toxin_and_protoxin_and_Cry2A_protoxin_on_the_predator_Chrysoperla_carnea

Dutton, A.; Klein, H.; Romeis, J.; Bigler, F. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperia carnea*. *Ecological Entomology*, 27: 441-7.

1. *Chrysoperla carnea* is an important predatory insect in maize. To assess the ecological effects of Bt-maize, expressing the Cry1Ab protein, on larvae of this predator, the following factors were examined: (1) the performance of three prey herbivores (*Rhopalosiphum padi*, *Tetranychus urticae*, and *Spodoptera littoralis*) on transgenic Bt and non-transgenic maize plants; (2) the intake of the Cry1Ab toxin by the three herbivores; and (3) the effects on *C. carnea* when fed each of the prey species.
2. The intrinsic rate of natural increase (r_m) was used as a measure of performance for *R. padi* and *T. urticae*. No difference in this parameter was observed between herbivores reared on Bt or non-transgenic plants. In contrast, a higher mortality rate and a delay in development were observed in *S. littoralis* larvae when fed Bt-maize compared with those fed the control maize plants.
3. The ingestion of Cry1Ab toxin by the different herbivores was measured using an immunological assay (ELISA). Highest amounts of Cry1Ab toxin were detected in *T. urticae*, followed by *S. littoralis*, and only trace amounts detected in *R. padi*.
4. Feeding *C. carnea* with *T. urticae*, which were shown to contain the Cry1Ab toxin, or with *R. padi*, which do not ingest the toxin, did not affect survival, development, or weight of *C. carnea*. In contrast, a significant increase in mortality and a delay in development were observed when predators were fed *S. littoralis* larvae reared on Bt-maize.
5. A combined interaction of poor prey quality and Cry1Ab toxin may account for the negative effects observed on *C. carnea* when fed *S. littoralis*. The relevance of these findings to the ecological risks of Bt-maize on *C. carnea* is discussed.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2311.2002.00436.x/pdf

Ponsard, S.; Gutierrez, A.; Mills, N. 2002. Effect of Bt-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four *heteropteran* predators. *Environmental Entomology*, 31: 1197-1205.

The management of agroecosystems affects intricately linked assemblages of organisms, and nontarget species are not necessarily unimpacted. We examined the effect of *Bt*-cotton and of lepidopteran prey (*Spodoptera exigua* Hübner) that had ingested it on the adult survivorship of four important heteropteran predators of cotton pests. Longevity significantly decreased for *Orius tristicolor* White and *Geocoris punctipes* Say (by 28 and 27% of the control value, respectively), whereas no effect was found for *Nabis* sp. and *Zelus renardii* Kolenati. This finding contrasts with the results of previous studies in which *Orius* spp. and *G. punctipes* were either fed only plant material or nonlepidopteran prey. *S. exigua* is a lepidopteran with low susceptibility to the *Bt* toxin expressed in cotton and therefore exemplifies the possible effect on predators of lepidopteran pests that would become resistant to *Bt.* The importance of *Bt* toxin type, the difference between plants and prey and between different prey species as routes of ingestion of *Bt* toxins, and the need for studies assessing the population and ecosystem-level effects of *Bt* cotton are discussed.

http://www.bioone.org/doi/abs/10.1603/0046-225X-31.6.1197?journalCode=enve

Baur, M.; Boethel, D. 2003. Effect of Bt-cotton expressing Cry1A(c) on the survival and fecundity of two *hymenopteran* parasitoids (*Braconidae*, *Encyrtidae*) in the laboratory. *Biological Control*, 26, 325–332.

We examined the effect of Bt-cotton (Event 531) plants expressing the *Bacillus thuringiensis* δ-endotoxin CryIA(c) on two hymenopteran endoparasitoids, *Cotesia marginiventris* and *Copidosoma floridanum*. In the laboratory, parasitized and unparasitized *Pseudoplusia includens* larvae were reared on foliage from a conventional soybean cultivar (Pioneer 97B61), a conventional cotton cultivar (DPL 5415), or a Bt-cotton cultivar (NuCotn 33B). *C. marginiventris* developed significantly faster within *P. includens* larvae feeding on Pioneer 97B61 and DPL 5415 compared to those feeding on NuCotn 33B. *C. marginiventris* that developed inside *P. includens* larvae feeding on NuCotn 33B suffered reduced longevity, and females had fewer ova. NuCotn 33B also affected the growth and development of *P. includens* parasitized with *C. floridanum* and life history parameters of adult *C. floridanum*. Parasitized and unparasitized *P. includens* developed more slowly when they were fed NuCotn 33B and the prepupae weighed less. Survival of parasitized and unparasitized *P. includens* was lower when larvae were fed NuCotn 33B and some evidence points to higher susceptibility of parasitized caterpillars to intoxication by NuCotn 33B. Fewer *C. floridanum* adults emerged from hosts fed NuCotn 33B, but pupal weight and adult longevity were unaffected. Analysis comparing the two experiments conducted with *C. floridanum* suggests that older NuCotn 33B plants (90–120 days after planting) may affect parasitoid development and adult survival less than younger NuCotn 33B plants (60–90 days after planting). Feeding on NuCotn 33B by *P. includens* affected the survival and development of the two hymenopteran endoparasitoids studied here, and the degree of the effect was similar to that observed with natural resistance found in soybean plants. It remains to be determined if the effects demonstrated here are less than, equal to, or greater than the impact of conventional insecticide applications used in conventional, non-transgenic, cotton.

http://www.sciencedirect.com/science/article/pii/S1049964402001603

REV – Lövei, G.; Arpaia, S. 2005. The impact of transgenic plants on natural enemies: a critical review of laboratory studies. *Entomologia Experimentalis et Applicata*, 114 (1):114.

We reviewed laboratory tests which studied the impact of genetically modified plants on arthropod natural enemies. A total of 18 species of predators and 14 species of parasitoids have been tested, most in only a few experiments. Certain groups (braconid wasps) or species (the green lacewing, *Chrysoperla carnea*) have attracted much effort, while representatives of others, including whole orders (e.g., Diptera), have never had a species tested. We conclude that laboratory tests are not the 'worst case' scenarios intended by the experimental designs, and are not often ecologically realistic: they typically provided ad libitum feeding, no prey choice, single prey type, no combination of stress factors and usually uniform temperatures. None of these are representative of field conditions, yet most could be easily mimicked in more complex laboratory tests. In most cases (94.6%), the studies were unable to indicate the level of power required to detect any impact. Small sample size and large variability are factors that mask all but very large differences in potential effects. For predators, 126 parameters were quantified, most commonly including survival/mortality (37 cases), development time (22), and body mass/size (20). For parasitoids, 128 parameters were quantified, the majority involving lectins or proteinase inhibitors. Most frequent measurements were: fecundity (23 experiments), adult longevity, extent of parasitism (17 each), body size, mortality, and larval development time. An aggregative scoring (summarising all quantified parameters) indicated that the laboratory tests quantified a remarkable number of cases (30% for predators, 39.8% for parasitoids), where the impacts of the genetically modified plant were significantly negative. These involve various parameters, organisms, test methods, and significance levels, but collectively they indicate that the use of genetically modified crops may result in negative effects on the natural enemies of crop pests.

http://onlinelibrary.wiley.com/doi/10.1111/j.0013-8703.2005.00235.x/abstract

Pilcher, C.; Rice, M.; Obrycki, J. 2005. Impact of transgenic *Bacillus thuringiensis* corn and crop phenology on five nontarget arthropods. *Environmental Entomology*, 34(5): 1302-1316.

Large-scale field studies were conducted to determine if temporal plantings of *Bacillus thuringiensis* (Berliner) (*Bt*) corn (event 176 and *Bt*11) would affect the seasonal abundance of the following generalist predators: *Coleomegilla maculata* DeGeer and *Cycloneda munda* (Say) (Coleoptera: Coccinellidae), *Orius insidiosus* (Say) (Heteroptera: Anthocoridae), *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and one specialist parasitoid, *Macrocentrus cingulum* Brischke (Hymenoptera: Braconidae). Adult populations were monitored using Pherocon AM yellow sticky traps at three locations in Iowa (1996–1998). At each location, a split-plot design was used with *Bt* and non-*Bt* corn as main plots and three planting dates as the split plots. Few differences in abundance were observed between *Bt* and non-*Bt* corn for the generalist predators studied. However, *M. cingulum*, a specialist parasitoid of European corn borer, was significantly affected by the presence of *Bt* corn. Densities of adult *M. cingulum* were 29–60% lower in *Bt* corn compared with non-*Bt* corn. Regression analyses indicated *M. cingulum* adults were preferentially recruited to and subsequently increased over time in the non-*Bt* corn treatments at each location within each year. Significant differences were observed among planting dates for all five species. Abundance effects from *Bt* corn on these natural enemies were not unexpected given the foraging and searching behaviors of different species and their varying levels of dependence on the presence of European corn borer.

Artigo completo disponível em http://ee.oxfordjournals.org/content/ee/34/5/1302.full.pdf

Liu, X-X.; Sun, C-G.; Zhang, Q-W. 2005. Effects of transgenic *Cry1A+CpTI* cotton and Cry1Ac toxin on the parasitoid, *Campoketis chlorideae* (*Hymenoptera*: *Ichneumonidae*). *Insect Science*, 12: 101-107.

The effect of transgenic double genes, *Cry1A+CpTI* cotton and Cry1Ac toxin on the parasitoid, *Campoketis chlorideae* Uchida of cotton bollworm, *Helicoverpa armigera* (Hübner), was investigated in the laboratory. *Helicoverpa armigera* larvae when in the first, second and third instar could not survive if fed on transgenic cotton leaves. Consequently, *C. chlorideae* larvae could not complete their development if parasitizing on such hosts. After *H. armigera* larvae were reared on transgenic or traditional cotton leaves for 12J48 hours, they were parasitized by *C. chlorideae* females. Parasitized larvae continued to feed on transgenic or traditional cotton for 12–48 h. The present results showed that the body weight of larvae of the parasitoids were significantly reduced when parasitized hosts fed on transgenic cotton leaves compared to those fed on traditional cotton. Duration of egg and larvae stage were significantly prolonged, pupal and adult weight of *C. chloridae* was decreased when the host larvae fed on transgenic cotton leaves longer than 48 h. The development duration of *C. chlorideae* pupae on the hosts fed on transgenic cotton leaves in each treatment was not significantly different from those of controls. The longevity of parasitoid females and males fed with a solution containing Cry1Ac toxin was not significantly different with that of the control.

http://onlinelibrary.wiley.com/doi/10.1111/j.1744-7917.2005.00012.x/abstract

Liu, X.; Zhang, Q.; Zhao, J-Z.; Cai, Q.; Xu, H.; Li, J. 2005. Effects of the Cry1Ac toxin of *Bacillus thuringiensis* on *Microplitis mediator*, a parasitoid of the cotton bollworm, *Helicoverpa armigera*. *Entomologia Experimentalist et Applicata*, 114: 205-213.

Interactions between the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), its larval parasitoid *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae), and the Cry1Ac toxin of *Bacillus thuringiensis* Berliner were evaluated under laboratory conditions. The growth of *H. armigera* larvae was delayed and its pupal rate and pupal weight decreased when they were fed on a diet containing Cry1Ac toxin. Due to the lowered growth rate of the host larvae, the time available for parasitization of *H. armigera* by *M. mediator* increased when the host larvae were reared on a diet containing Cry1Ac toxin at concentrations of 0.5, 1, 2, and 4 μg g^{-1}. The longevity of female and male parasitoids was not significantly affected when newly emerging wasps fed on honey solutions containing three different concentrations of Cry1Ac toxin (125, 250, and 500 μg ml^{-1}). When female parasitoids were fed on honey solutions containing Cry1Ac, their offsprings' egg and larval development period, pupal weight, length of pupation, adult weight, and adult longevity did not change significantly in most of the treatments compared with controls. When the female parasitoids parasitized host larvae that had been fed on a diet containing 0.5, 1, 2, 4, and 8 μg g^{-1} Cry1Ac toxin, their offsprings' eggs and larvae were significantly delayed. Their pupal weight, adult weight, and adult longevity were also significantly less than controls.

http://onlinelibrary.wiley.com/doi/10.1111/j.1570-7458.2005.00248.x/abstract

Zhang, G-F.; Wan, F-H.; Lövei, G.; Liu, W-X.; Guo, J-Y. 2006. Transmission of Bt toxin to the predator *Propylaea japonica* (*Coleoptera*: *Coccinellidae*) through its aphid prey feeding on transgenic Bt cotton. *Environmental Entomology*, 35(1): 143-150.

Laboratory feeding experiments using transgenic *Bacillus thuringiensis* (*Bt*) cotton plants were carried out to evaluate the transmission of *Bt* toxin among trophic levels and the effects of *Bt*-fed herbivorous prey on the coccinellid predator *Propylaea japonica* (Thunberg). The experimental host plants were transgenic *Bt*-expressing cotton cultivars, NuCOTN 33B and GK-12 and one corresponding untransformed isogenic (non-*Bt*) cultivar. The herbivorous prey, cotton aphid *Aphis gossypii* Glover, was not sensitive to *Bt* toxin. Trace amounts of *Bt* toxins (6.0 ng/g fresh mass [FM] in GK-12, 4.0 ng/g FM in NuCOTN 33B) were detected in *A. gossypii* feeding on *Bt* cotton cultivars. *Bt* toxin was detected in ladybirds preying on *Bt*-fed aphids, and its quantity increased as the predatory period extended (5–20 d). Small amounts of *Bt* toxin was also found in newly hatched, unfed coccinellid larvae when their parents fed on NuCOTN 33B-reared aphids (15.0 ng/g FM), but not when the parents were fed on GK-12–reared prey. In experiments assessing life history consequences, mortality was low (mean = 7.9%), confirming that the rearing methods were appropriate. There were no distinct differences in preimaginal mortality between predators reared on *Bt*-fed or *Bt*-free aphids. The preimaginal stages of the ladybird beetles developed faster when reared on prey fed on either *Bt*-cotton cultivar than those fed control prey. There was a trend of more adult malformations when the predator was fed with prey from one (GK-12) but not the other of the *Bt* cotton cultivars than on control prey. There were no significant differences in the preovipositing period or in fecundity. Ladybird beetles preying on *Bt*-reared aphids matured faster and mated more frequently than those fed on *Bt*-free aphids. These results indicate that *Bt* toxin expressed in transgenic cotton cultivars can be transmitted to a higher trophic level through a nontarget pest insect and may alter the biology and behavior of a predatory ladybird. Further work should evaluate the possible long-term, sublethal impacts on the agroenvironment under field conditions.

http://www.bioone.org/doi/abs/10.1603/0046-225X-35.1.143?journalCode=enve

REV – Lövei, G.; Andow, D.; Arpaia, S. 2009. Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. *Environmental Entomology*, 38(2): 293-306.

This review uses a data-driven, quantitative method to summarize the published, peer-reviewed literature about the impact of genetically modiÞed (GM) plants on arthropod natural enemies in laboratory experiments. The method is similar to meta-analysis, and, in contrast to a simple author-vote counting method used by several earlier reviews, gives an objective, data-driven summary of existing knowledge about these effects. SigniÞcantly more non-neutral responses were observed than expected at random in 75% of the comparisons of natural enemy groups and response classes. These observations indicate that Cry toxins and proteinase inhibitors often have non-neutral effects on natural enemies. This synthesis identiÞes a continued bias toward studies on a few predator species, especially the green lacewing, *Chrysoperla carnea* Stephens, which may be more sensitive to GM insecticidal plants (16.8% of the quantified parameter responses were significantly negative) than predators in general (10.9% significantly negative effects without *C. carnea*). Parasitoids were more susceptible than predators to the effects of both Cry toxins and proteinase inhibitors, with fewer positive effects (18.0%, significant and nonsignificant positive effects combined) than negative ones (66.1%, significant and nonsignificant negative effects combined).GMplants can have a positive effect on natural enemies (4.8% of responses were significantly positive), although significant negative (21.2%) effects were more common. Although there are data on 48 natural enemy species, the database is still far from adequate to predict the effect of a *Bt* toxin or proteinase inhibitor on natural enemies.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/ReviewLoeveietal.pdf

Zhou, J.; Xiao, K.; Wei, B.; Wang, Z.; Tian, Y.; Tian, Y.; Song, Q. 2014. Bioaccumulation of Cry1Ab protein from an herbivore reduces anti-oxidant enzyme activities in two spider species. *PLOS ONE*, 9(1): e84724. doi:10.1371/journal.pone.0084724.

Cry proteins are expressed in rice lines for lepidopteran pest control. These proteins can be transferred from transgenic rice plants to non-target arthropods, including planthoppers and then to a predatory spider. Movement of Cry proteins through food webs may reduce fitness of non-target arthropods, although recent publications indicated no serious changes in non-target populations. Nonetheless, Cry protein intoxication influences gene expression in Cry-sensitive insects. We posed the hypothesis that Cry protein intoxication influences enzyme activities in spiders acting in tri-trophic food webs. Here we report on the outcomes of experiments designed to test our hypothesis with two spider species. We demonstrated that the movement of CryAb protein from *Drosophila* culture medium into fruit flies maintained on the CryAb containing medium and from the flies to the spiders *Ummeliata insecticeps* and *Pardosa pseudoannulata*. We also show that the activities of three key metabolic enzymes, acetylcholine esterase (AchE), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) were significantly influenced in the spiders after feeding on Cry1Ab-containing fruit flies. We infer from these data that Cry proteins originating in transgenic crops impacts non-target arthropods at the physiological and biochemical levels, which may be one mechanism of Cry protein-related reductions in fitness of non-target beneficial predators.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0084724

1.3 Dispersão e persistência das proteínas Bt no meio ambiente e desequilíbrios por elas causados nas comunidades da microbiota do solo

Como exemplificado em artigos acima referenciados, as proteínas Cry se dispersam e permanecem por tempos significativos em diversos ambientes: cadeias alimentares, solo, corpos d’água interconectados com as lavouras, ecossistemas naturais e seminaturais dos entornos da lavoura, etc.

Exsudações de proteínas Cry, pelas raízes das plantas Bt, disseminação de pólen e biotransposição das toxinas ao longo das redes alimentares (inclusive na esfera dos decompositores) incluem-se entre os principais meios de dispersão dessas proteínas inseticidas no meio ambiente.

1.3.1 Disseminação e persistência das toxinas Bt nas cadeias tróficas

Dutton, A.; Klein, H.; Romeis, J.; Bigler, F. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperia carnea*. *Ecological Entomology*, 27: 441-7.

1. *Chrysoperla carnea* is an important predatory insect in maize. To assess the ecological effects of Bt-maize, expressing the Cry1Ab protein, on larvae of this predator, the following factors were examined: (1) the performance of three prey herbivores (*Rhopalosiphum padi*, *Tetranychus urticae*, and *Spodoptera littoralis*) on transgenic Bt and non-transgenic maize plants; (2) the intake of the Cry1Ab toxin by the three herbivores; and (3) the effects on *C. carnea* when fed each of the prey species.
2. The intrinsic rate of natural increase (r_m) was used as a measure of performance for *R. padi* and *T. urticae*. No difference in this parameter was observed between herbivores reared on Bt or non-transgenic plants. In contrast, a higher mortality rate and a delay in development were observed in *S. littoralis* larvae when fed Bt-maize compared with those fed the control maize plants.
3. The ingestion of Cry1Ab toxin by the different herbivores was measured using an immunological assay (ELISA). Highest amounts of Cry1Ab toxin were detected in *T. urticae*, followed by *S. littoralis*, and only trace amounts detected in *R. padi*.
4. Feeding *C. carnea* with *T. urticae*, which were shown to contain the Cry1Ab toxin, or with *R. padi*, which do not ingest the toxin, did not affect survival, development, or weight of *C. carnea*. In contrast, a significant increase in mortality and a delay in development were observed when predators were fed *S. littoralis* larvae reared on Bt-maize.
5. A combined interaction of poor prey quality and Cry1Ab toxin may account for the negative effects observed on *C. carnea* when fed *S. littoralis*. The relevance of these findings to the ecological risks of Bt-maize on *C. carnea* is discussed.

http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2311.2002.00436.x/abstract

Harwood, J.; Wallin, W.; Obrycki, J. 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. *Molecular Ecology*, 14, 2815–2823.

The planting of transgenic crops expressing Bacillus thuringiensis endotoxins is widespread throughout the world; the prolific increase in their application exposes nontarget organisms to toxins designed to control pests. To date, studies have focused upon the effects of Bt endotoxins on specific herbivores and detritivores, without consideration of their persistence within arthropod food webs. Here, we report the first quantitative field evaluation of levels of Bt endotoxin within nontarget herbivores and the uptake by higher order arthropods. Antibody-based assays indicated significant quantities of detectable Cry1Ab endotoxin within nontarget herbivores which feed on transgenic corn (including the corn flea beetle, Chaetocnema pulicaria, Japanese beetle, Popillia japonica and southern corn rootworm, Diabrotica undecimpunctata howardi). Furthermore, arthropod predators (Coccinellidae, Araneae, and Nabidae) collected from these agroecosystems also contained significant quantities of Cry1Ab endotoxin indicating its movement into higher trophic levels. This uptake by predators is likely to have occurred by direct feeding on plant material (in predators which are facultatively phytophagous) or the consumption of arthropod prey which contained these proteins. These data indicate that long-term exposure to insecticidal toxins occurs in the field. These levels of exposure should therefore be considered during future risk assessments of transgenic crops to nontarget herbivores and arthropod predators.

http://www.ncbi.nlm.nih.gov/pubmed/16029480

Obrist, L.; Dutton, A.; Albajes, R.; Bigler, F. 2006. Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. *Ecological Entomology*, 31, 143–154.

1. To assess the risks of an insect-resistant transgenic plant for non-target arthropods, it is important to investigate the exposure of non-target species to the transgene product. Exposure of predators in the field depends on the toxin levels in food sources, their feeding ecology and that of their prey.
2. To verify the transmission of Cry1Ab toxin through the food chain, and thus exposure of predators in the field, samples from different plant tissues, herbivores, and predators in Bt maize fields in Spain (Event 176) were collected at different periods over the season and the toxin content was measured using ELISA. Complementary laboratory studies were performed with the omnivorous predator *Orius majusculus* to assess the toxin uptake and persistence after feeding on variable Bt-containing food sources.
3. Field results revealed that toxin content in some herbivores was negligible (aphids, thrips, leafhoppers) compared with those in spider mites. The latter herbivore only occurred after pollen shed and contained three times greater toxin levels than Bt maize leaves.
4. Data confirmed that the Bt toxin can be transferred to predators, that is to say to *Orius* spp., *Chrysoperla* spp., and *Stethorus* sp. This only applied when Bt maize pollen or spider mites were available. The passage of Bt toxin to *O. majusculus* via these two food sources was also confirmed in the laboratory. Contrastingly, some predators in the field (hemerobiids, *Nabis* sp., *Hippodamia* sp., *Demetrias* sp.) contained no or negligible toxin levels even when pollen or spider mites were present.
5. Besides essential information for exposure assessment of numerous arthropod predators, this study provides an insight into the feeding ecology of different arthropods in the maize system.

http://onlinelibrary.wiley.com/doi/10.1111/j.0307-6946.2006.00762.x/abstract

Obrist, L.; Dutton, A.; Romeis, J.; Bigler, F. 2006. Biological activity of Cry1Ab toxin expressed by Bt maize following ingestion by herbivorous arthropods and exposure of the predator *Chrysoperla carnea*. *BioControl*, 51: 31-48.

A major concern regarding the deployment of insect resistant transgenic plants is their potential impact on non-target organisms, in particular on beneficial arthropods such as predators. To assess the risks that transgenic plants pose to predators, various experimental testing systems can be used. When using tritrophic studies, it is important to verify the actual exposure of the predator, i.e., the presence of biologically active toxin in the herbivorous arthropod (prey). We therefore investigated the uptake of Cry1Ab toxin by larvae of the green lacewing (Chrysoperla carnea (Stephens); Neuroptera: Chrysopidae) after consuming two Bt maize-fed herbivores (Tetranychus urticae Koch; Acarina: Tetranychidae and Spodoptera littoralis (Boisduval); Lepidoptera: Noctuidae) by means of an immunological test (ELISA) and the activity of the Cry1Ab toxin following ingestion by the herbivores. Moreover, we compared the activity of Cry1Ab toxin produced by Bt maize to that of purified toxin obtained from transformed Escherichia coli, which is recommended to be used in toxicity studies. The activity of the toxin was assessed by performing feeding bioassays with larvae of the European corn borer (Ostrinia nubilalis (Hübner); Lepidoptera: Crambidae), the target pest of Cry1Ab expressing maize. ELISA confirmed the ingestion of Bt toxin by C. carnea larvae when fed with either of the two prey species and feeding bioassays using the target pest showed that the biological activity of the Cry1Ab toxin is maintained after ingestion by both herbivore species. These findings are discussed in the context of previous risk assessment studies with C. carnea. The purified Cry1Ab protein was more toxic to O. nubilalis compared to the plant-derived Cry1Ab toxin when applied at equal concentrations according to ELISA measurements. Possible reasons for these findings are discussed.

http://link.springer.com/article/10.1007%2Fs10526-005-2936-8

Harwood, J.; Samson, R.; Obrycki, J. 2007. Temporal detection of Cry1Ab-endotoxins in *coccinellid* predators from fields of *Bacillus thuringiensis* corn. *Bull Entomol Res*, 97(6):643-8.

The area planted to genetically engineered crops has increased dramatically in the last ten years. This has generated many studies examining non-target effects of bioengineered plants expressing Bacillus thuringiensis endotoxins. To date, most have focused on population-level effects in the field or laboratory evaluation of specific plant-herbivore or plant-herbivore-predator trophic pathways. Using a post-mortem enzyme-linked immunosorbent assay, we examined the uptake of Cry1Ab-endotoxins by predatory coccinellids and the importance of anthesis to this trophic pathway. Adult Coleomegilla maculata, Harmonia axyridis, Cycloneda munda and Coccinella septempunctata contained low, but detectable, quantities of Bt-endotoxin when screened by ELISA. This was most evident in C. maculata, with 12.8% of 775 individuals testing positive for Cry1Ab-endotoxins. Interestingly, the presence of endotoxins in gut samples was not confined to periods around anthesis, but coccinellid adults tested positive two weeks before and up to ten weeks after pollen was shed, suggesting tri-trophic linkages in their food chain facilitates the transfer of endotoxins into higher-order predators. This contrasts with adult Coleomegilla maculata entering overwintering sites where Bt-endotoxins were not detected in gut samples, indicating low levels of persistence of Cry1Ab-endotoxins within coccinellid predators. This study enhances our understanding of complex interactions between transgenic crops and non-target food webs, but further research is required to quantify the significance of specific trophic linkages in the field.

http://www.ncbi.nlm.nih.gov/pubmed/17997879

Douville, M.; Gagné, F.; André, C; Blaise, C. 2009. Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (*Elliptio complanata*) near corn fields: evidence of exposure by bacterial ingestion. *Ecotoxicology and Environmental Safety*, 72: 17-25.

The purpose of this study was to examine the contamination of cry1 and cry1Ab genes from Bacillus thuringiensis and transgenic corn in feral freshwater mussels collected from sites located in proximity of corn fields. In addition, mussels were transplanted for 2 months to a site in the Huron River, upstream to the Richelieu River, which is subject to intensive corn farming. Mussels were significantly contaminated by both genes in their gills, digestive glands, and gonads, as determined by qPCR methodology. Gene sequence analysis confirmed the presence of transgenic corn cry1Ab gene in mussel tissues. In an attempt to explain the presence of the transgene in mussel tissues, heterotrophic bacteria were grown from surface water and sediment samples on agar plates in the Richelieu River in May and August. The transgene was found at two out of six surface water samples and in one sediment sample. The study revealed that exposure to transgenic corn cry1Ab gene in mussels seems to proceed by ingestion of microorganisms during feeding.

Artigo completo disponível em http://www.fondazionedirittigenetici.org/fondazione/files/maismolluschi.pdf

Zurbrügg, C.; Nentwig, W. 2009. Ingestion and excretion of two transgenic Bt corn varieties by slugs. *Transgenic Res*, 18(2):215-25. doi: 10.1007/s11248-008-9208-1.

The release of transgenic Bacillus thuringiensis (Bt) corn expressing various Cry endotoxins has raised concern that these endotoxins are disseminated in the food web and may adversely affect non-target beneficial organisms, such as predators and organisms of the decomposer food web. We therefore investigated in a laboratory study, whether the Cry1Ab and Cry3Bb1 protein from Bt corn could potentially be transferred to such organisms by measuring the Cry protein content in the two common agricultural slug pests Arion lusitanicus and Deroceras reticulatum and their feces. We measured Cry1Ab and Cry3Bb1 protein concentration in leaves, intestines, and feces of corn leaf-fed slugs using ELISA and determined how much of the ingested protein is excreted by the slugs. Cry3Bb1 concentration in leaves of DKC5143Bt corn was significantly higher than Cry1Ab concentration in leaves of N4640Bt corn. While slugs were feeding on corn leaves, the Cry3Bb1 and Cry1Ab proteins were found in intestines and feces of both slug species. Bt protein concentrations in intestines of Cry3Bb1 corn-fed slugs were in both slug species higher than in Cry1Ab corn fed slugs, whereas no differences between Cry3Bb1 and Cry1Ab protein in feces were found. After slugs had ceased feeding on Bt corn, Cry1Ab was detectable in fresh slug feces for a significantly longer time and often in higher amounts than the Cry3Bb1. Our results indicate that both Cry proteins are likely to be transferred to higher trophic levels and to the decomposer food web. Since different Bt proteins seem to vary in their degradation, they have different transfer probabilities. This should be considered in risk assessments for non-target arthropods.

http://www.ncbi.nlm.nih.gov/pubmed/18763046

Yu, H.; Romeis, J.; Li, Y.; Li, X.; Wu, K. 2014. Acquisition of Cry1Ac protein by non-target arthropods in Bt soybean fields. *PLOS ONE*, 9(8): e103973. Doi:10.1371/journal.pone.0103973.

Soybean tissue and arthropods were collected in *Bt* soybean fields in China at different times during the growing season to investigate the exposure of arthropods to the plant-produced Cry1Ac toxin and the transmission of the toxin within the food web. Samples from 52 arthropod species/taxa belonging to 42 families in 10 orders were analysed for their Cry1Ac content using enzyme-linked immunosorbent assay (ELISA). Among the 22 species/taxa for which three samples were analysed, toxin concentration was highest in the grasshopper *Atractomorpha sinensis* and represented about 50% of the concentration in soybean leaves. Other species/taxa did not contain detectable toxin or contained a concentration that was between 1 and 10% of that detected in leaves. These Cry1Ac-positive arthropods included a number of mesophyll-feeding Hemiptera, a cicadellid, a curculionid beetle and, among the predators, a thomisid spider and an unidentified predatory bug belonging to the Anthocoridae. Within an arthropod species/taxon, the Cry1Ac content sometimes varied between life stages (nymphs/larvae *vs.* adults) and sampling dates (before, during, and after flowering). Our study is the first to provide information on Cry1Ac-expression levels in soybean plants and Cry1Ac concentrations in non-target arthropods in Chinese soybean fields. The data will be useful for assessing the risk of non-target arthropod exposure to Cry1Ac in soybean.

Artigo completo disponível em http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0103973

1.3.2 Disseminação e persistência das toxinas Bt no meio ambiente (solo e água)

Estudos mostram que as toxinas permanecem ativas no solo por períodos variáveis, expandidos em terrenos argilosos e na presença de agroquímicos relacionados a plantas transgênicas que carregam tolerância ao glifosato e a outros herbicidas.

Koskella, J.; Stotzky, G. 1997. Microbial utilization of free and clay-bound insecticidal toxins from *Bacillus thuringiensis* and their retention of insecticidal activity after incubation with microbes. *Applied and Environmental Microbiology*, 1997, p. 3561–3568.

The insecticidal toxins produced by Bacillus thuringiensis subspp. kurstaki and tenebrionis were resistant when bound on clays, but not when free, to utilization by pure and mixed cultures of microbes as sources of carbon and carbon plus nitrogen, and their availability as a nitrogen source was reduced. The bound toxins retained insecticidal activity both before and after exposure to microbes or pronase. The insecticidal activity of the toxins persisted for 40 days (the longest time evaluated) in nonsterile soil continuously maintained at the -33-kPa water tension and room temperature, alternately air dried and rewetted to the -33-kPa water tension, or alternately frozen and thawed, although alternate drying and wetting reduced the activity.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1389248/pdf/hw3561.pdf

Tapp, H.; Stozsky, G. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology Biochemistry*, 30 (4): 471-476.

The accumulation and persistence of the insecticidal toxins from *Bacillus thuringiensis* may result in environmental hazards, such as toxicity to nontarget species and the selection of toxin-resistant target species. Toxins from *B. thuringiensis* subsp. *kurstaki* were added to three soils [Kitchawan soil (which contains kaolinite but not montmorillonite) unamended or amended with montmorillonite or kaolinite (as an internal control); Mopala soil, which contains montmorillonite and kaolinite; and San Alejo soil, which does not contain montmorillonite but contains kaolinite], and the persistence of the toxins was determined by insect bioassays using the larvae of the tobacco hornworm (*Manduca sexta*). Toxicity varied with the type of soil: the Kitchawan soil, either unamended or amended with kaolinite, remained toxic to the larvae for more than 6 months, maintaining a lethal concentration at which 50% of the larvae were killed (LC_{50}) of 61 to 111 ng 100 μl^{-1} of soil suspension throughout 195 d of incubation. The Kitchawan soil amended with montmorillonite and the Mopala and San Alejo soils showed reduced insecticidal activity after only 35 d (LC_{50} from 104 to 192 ng 100 μl^{-1}). The pH of soils in which insecticidal activity was reduced was higher (5.8 to 7.3) than that of soils in which insecticidal activity was retained (4.9 to 5.1). As microbial activity is greater at higher pH values, more of the toxins may have been degraded by microbes in soils with the higher pH values. This hypothesis was confirmed by the greater loss in insecticidal activity during 234 d when the pH of the Kitchawan soil, unamended or amended to 6% (vol vol^{-1}) with kaolinite, was increased from 4.9 to *ca.* 7.0 by the addition of $CaCO_3$.

http://www.sciencedirect.com/science/article/pii/S003807179700148X

COM – Saxena, D.; Flores, S.; Stozskyk, G. 1999. Transgenic plants: Insecticidal toxin in root exudates from Bt corn. *Nature*, 402: 480.

Bt corn is corn (*Zea mays*) that has been genetically modified to express insecticidal toxins derived from the bacterium *Bacillus thuringiensis* to kill lepidopteran pests feeding on these plants. Here we show that *Bt* toxin is released into the rhizosphere soil in root exudates from *Bt* corn.

http://www.nature.com/nature/journal/v402/n6761/abs/402480a0.html#close

Saxena, D.; Stotzky, G. 2000. Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn in vitro and in situ. *FEMS Microbiology Ecology*, 33(1):35-39.

The insecticidal toxin encoded by the cry1Ab gene from Bacillus thuringiensis was released in root exudates from transgenic Bt corn during 40 days of growth in soil amended to 0, 3, 6, 9, or 12% (v/v) with montmorillonite or kaolinite in a plant growth room and from plants grown to maturity in the field. The presence of the toxin in rhizosphere soil was determined by immunological and larvicidal assays. No toxin was detected in any soils from isogenic non-Bt corn or without plants. Persistence of the toxin was apparently the result of its binding on surface-active particles in the soils, which reduced the biodegradation of the toxin. The release of the toxin could enhance the control of insect pests or constitute a hazard to nontarget organisms, including the microbiota of soil, and increase the selection of toxin-resistant target insects.

Artigo completo disponível em http://femsec.oxfordjournals.org/content/33/1/35.long

Saxena, D.; Flores, S.; Stotzky, G. 2002. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil Biology and Biochemistry*, Volume 34, Issue 1, Pages 133–137.

The anti-lepidopteran toxin (Cry1Ab protein) encoded by truncated genes from *Bacillus thuringiensis* was released in the root exudates from all hybrids of Bt corn studied and which represented three transformation events (Bt11, MON810, and 176). In vitro and in situ studies indicated that the toxin released in root exudates accumulates in soil, as it adsorbs and binds rapidly on surface-active particles (e.g. clays and humic substances), and retains insecticidal activity for at least 180 d, the longest time studied. The results indicated that the release of the Cry1Ab protein by roots is a common phenomenon with transgenic Bt corn and is not restricted to only the one Bt corn hybrid (NK4640Bt) and tranformation event (Bt11) studied initially.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/SAXENAsoil.pdf

Zwahlen, C.; Hilbeck, A.; Gugerli, P.; Nentwig, W. 2003. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. *Molecular Ecology*, 12 (3): 765-775.

Large quantities of Bacillus thuringiensis (Bt) corn plant residue are left in the field after harvest, which may have implications for the soil ecosystem. Potential impacts on soil organisms will also depend on the persistence of the Bt toxin in plant residues. Therefore, it is important to know how long the toxin persists in plant residues. In two field studies in the temperate corn-growing region of Switzerland we investigated degradation of the Cry1Ab toxin in transgenic Bt corn leaves during autumn, winter and spring using an enzyme-linked immunosorbent assay (ELISA). In the first field trial, representing a tillage system, no degradation of the Cry1Ab toxin was observed during the first month. During the second month, Cry1Ab toxin concentrations decreased to approximately 20% of their initial values. During winter, there was no further degradation. When temperatures again increased in spring, the toxin continued to degrade slowly, but could still be detected in June. In the second field trial, representing a no-tillage system, Cry1Ab toxin concentrations decreased without initial delay as for soil-incorporated Bt plants, to 38% of the initial concentration during the first 40 days. They then continued to decrease until the end of the trial after 200 days in June, when 0.3% of the initial amount of Cry1Ab toxin was detected. Our results suggest that extended pre- and post-commercial monitoring are necessary to assess the long-term impact of Bt toxin in transgenic plant residues on soil organisms.

http://www.ncbi.nlm.nih.gov/pubmed/12675831

Lee, L.; Saxena, D.; Stotzky, G. 2003. Activity of free and clay-bound insecticidal proteins from *Bacillus thuringiensis* subsp. *israelensis* against the Mosquito *Culex pipiens. Applied and Environmental Microbiology*, vol. 69 no. 7 4111-4115.

Bacillus thuringiensis subsp. *israelensis* produces parasporal insecticidal crystal proteins (ICPs) that have larvicidal activity against some members of the order Diptera, such as blackflies and mosquitoes. Hydrolysis of the ICPs in the larval gut results in four major proteins with a molecular mass of 27, 65, 128, and 135 kDa. Toxicity is caused by synergistic interaction between the 25-kDa protein (proteolytic product of the 27-kDa protein) and one or more of the higher-molecular-mass proteins. Equilibrium adsorption of the proteins on the clay minerals montmorillonite and kaolinite, which are homoionic to various cations, was rapid (<30 min for maximal adsorption), increased with protein concentration and then reached a plateau (68 to 96% of the proteins was adsorbed), was significantly lower on kaolinite than on montmorillonite, and was not significantly affected by the valence of the cation to which the clays were homoionic. Binding of the toxins decreased as the pH was increased from 6 to 11, and there was 35 to 66% more binding in phosphate buffer at pH 6 than in distilled water at pH 6 or 7.2. Only 2 to 12% of the adsorbed proteins was desorbed by two washes with water; additional washings desorbed no more toxins, indicating that they were tightly bound. Formation of clay-toxin complexes did not alter the structure of the proteins, as indicated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the equilibrium supernatants and desorption washes and by dot blot enzyme-linked immunosorbent assay of the complexes, which was confirmed by enhanced chemiluminescence Western blot analysis. Free and clay-bound toxins resulted in 85 to 100% mortality of the mosquito *Culex pipiens*. Persistence of the bound toxins in nonsterile water after 45 days was significantly greater (mortality of 63% ± 12.7%) than that of the free toxins (mortality of 25% ± 12.5%).

Artigo completo disponível em http://aem.asm.org/content/69/7/4111.full

Saxena, D.; Stewart, C.; Altosaar, I.; Shu, Q.; Stotzky, G. 2004. Larvicidal cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco. *Plant Physiology & Biochemistry*, 42 (5): 383-387.

Larvicidal proteins encoded by cry genes from Bacillus thuringiensis were released in root exudates from transgenic B. thuringiensis corn, rice, and potato but not from B. thuringiensis canola, cotton, and tobacco. Nonsterile soil and sterile hydroponic solution in which B. thuringiensis corn, rice, or potato had been grown were immunologically positive for the presence of the Cry proteins; from B. thuringiensis corn and rice, the soil and solution were toxic to the larva of the tobacco hornworm (Manduca sexta), and from potato, to the larva of the Colorado potato beetle (Leptinotarsa decemlineata), representative lepidoptera and coleoptera, respectively. No toxin was detected immunologically or by larvicidal assay in soil or hydroponic solution in which B. thuringiensis canola, cotton, or tobacco, as well as all near-isogenic non-B. thuringiensis plant counterparts or no plants, had been grown. All plant species had the cauliflower mosaic virus (CaMV) 35S promoter, except rice, which had the ubiquitin promoter from maize. The reasons for the differences between species in the exudation from roots of the toxins are not known. The released toxins persisted in soil as the result of their binding on surface-active particles (e.g. clay minerals, humic substances), which reduced their biodegradation. The release of the toxins in root exudates could enhance the control of target insect pests, constitute a hazard to nontarget organisms, and/or increase the selection of toxin-resistant target insects.

http://www.ncbi.nlm.nih.gov/pubmed/15191740

Stotzky, G. 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil*, 266: 77- 89.

Insecticidal proteins produced by various subspecies (*kurstaki, tenebrionis*, and *israelensis*) of *Bacillus thuringiensis* (*Bt*) bound rapidly and tightly on clays, both pure mined clay minerals and soil clays, on humic acids extracted from soil, and on complexes of clay and humic acids. Binding reduced susceptibility of the proteins to microbial degradation. However, bound proteins retained biological activity. Purified Cry1Ab protein and protein released from biomass of transgenic *Bt* corn and in root exudates of growing *Bt* corn (13 hybrids representing three transformation events) exhibited binding and persistence in soil. Insecticidal protein was also released in root exudates of *Bt* potato (Cry3A protein) and rice (Cry1Ab protein) but not in root exudates of *Bt* canola, cotton, and tobacco (Cry1Ac protein). Vertical movement of CrylAb protein, either purified or in root exudates or biomass of *Bt* corn, decreased as the concentration of the clay minerals, kaolinite or montmorillonite, in soil increased.
Biomass of transgenic *Bt* corn decomposed less in soil than biomass of near-isogenic non-*Bt* corn, possibly because biomass of *Bt* corn had a significantly higher content of lignin than biomass of non-*Bt* corn. Biomass of *Bt* canola, cotton, potato, rice, and tobacco also decomposed less than biomass of the respective near-isogenic non-*Bt* plants. However, the lignin content of these *Bt* plants, whichwas significantly less than that of *Bt* corn, was not significantly different from that of their near-isogenic non-*Bt* counterparts, although it was consistently higher. The Cry1Ab protein had no consistent effects on organisms (earthworms, nematodes, protozoa, bacteria, fungi) in soil or *in vitro*. The Cry1Ab protein was not taken up from soil by non-*Bt* corn, carrot, radish, or turnip grown in soil in which *Bt* corn had been grown or into which biomass of *Bt* corn had been incorporated.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/webfm/plataforma/sbtbacillusthuringiensisfromtransgenicplantsstotzky.pdf

Baumgarte, S.; Tebbe, C. 2005. Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. *Molecular Ecology*, 14 (8): 2539 2551.

Field studies were done to assess how much of the transgenic, insecticidal protein, Cry1Ab, encoded by a truncated cry1Ab gene from Bacillus thuringiensis (Bt), was released from Bt-maize MON810 into soil and whether bacterial communities inhabiting the rhizosphere of MON810 maize were different from those of the rhizosphere of nontransgenic maize cultivars. Bacterial community structure was investigated by SSCP (single-strand conformation polymorphism) of PCR-amplified 16S rRNA genes from community DNA. Using an improved extraction and detection protocol based on a commercially available ELISA, it was possible to detect Cry1Ab protein extracted from soils to a threshold concentration of 0.07 ng/g soil. From 100 ng of purified Cry1Ab protein added per gram of soil, only an average of 37% was extractable. At both field sites investigated, the amount of Cry1Ab protein in bulk soil of MON810 field plots was always lower than in the rhizosphere, the latter ranging from 0.1 to 10 ng/g soil. Immunoreactive Cry1Ab protein was also detected at 0.21 ng/g bulk soil 7 months after harvesting, i.e. in April of the following year. At this time, however, higher values were found in residues of leaves (21 ng/g) and of roots (183 ng/g), the latter corresponding to 12% of the Cry1Ab protein present in intact roots. A sampling 2 months later indicated further degradation of the protein. Despite the detection of Cry1Ab protein in the rhizosphere of MON810 maize, the bacterial community structure was less affected by the Cry1Ab protein than by other environmental factors, i.e. the age of the plants or field heterogeneities. The persistence of Cry1Ab protein emphasizes the importance of considering post-harvest effects on nontarget organisms.

http://www.ncbi.nlm.nih.gov/pubmed/15969733

Rosi-Marshall, E.; Tank, J.; Royer, T.; Whiles, M.; Evans-White, M.; Chambers, C.; Griffiths, N.; Pokelsek, J.; Stephen, M. 2007. Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *PNAS*, 104(41): 16204-16208.

Corn (*Zea mays* L.) that has been genetically engineered to produce the Cry1Ab protein (Bt corn) is resistant to lepidopteran pests. Bt corn is widely planted in the midwestern United States, often adjacent to headwater streams. We show that corn byproducts, such as pollen and detritus, enter headwater streams and are subject to storage, consumption, and transport to downstream water bodies. Laboratory feeding trials showed that consumption of Bt corn byproducts reduced growth and increased mortality of nontarget stream insects. Stream insects are important prey for aquatic and riparian predators, and widespread planting of Bt crops has unexpected ecosystem-scale consequences.

Artigo completo disponível em http://www.pnas.org/content/104/41/16204.full

Douville, M.; Gagné, F.; Blaise, C.; André, C. 2007. Occurrence and persistence of *Bacillus thuringiensis* (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. *Ecotoxicology and Environmental Safety*, 66 (2): 195203.

Genetically modified corn crops and suspensions of Bacillus thuringiensis (Bt) are currently used to control pest infestations of insects of the Lepidoptera family. For this purpose, the cry1Ab gene coding for protein delta-endotoxin derived from B. thuringiensis kurstaki (Btk), which is highly toxic to these insects, was inserted and expressed in corn. The aims of this study were to examine the occurrence and persistence of the cry1Ab gene from Btk and Bt corn in aquatic environments near fields where Bt corn was cultivated. First, an optimal DNA preparation and extraction methodology was developed to allow for quantitative gene analysis by real-time polymerase chain reaction (qPCR) in various environmental matrices. Second, surface water and sediment were spiked in vitro with genomic DNA from Bt or Bt corn to evaluate the persistence of cry1Ab genes. Third, soil, sediment, and water samples were collected before seeding, 2 weeks after pollen release, and after corn harvesting and mechanical root remixing in soils to assess cry1Ab gene content. DNA was extracted with sufficient purity (i.e., low absorbance at 230 nm and absence of PCR-inhibiting substances) from soil, sediment, and surface water. The cry1Ab gene persisted for more than 21 and 40 days in surface water and sediment, respectively. The removal of bacteria by filtration of surface water samples did not significantly increase the half-life of the transgene, but the levels were fivefold more abundant than those in unfiltered water at the end of the exposure period. In sediments, the cry1Ab gene from Bt corn was still detected after 40 days in clay- and sand-rich sediments. Field surveys revealed that the cry1Ab gene from transgenic corn and from naturally occurring Bt was more abundant in the sediment than in the surface water. The cry1Ab transgene was detected as far away as the Richelieu and St. Lawrence rivers (82 km downstream from the corn cultivation plot), suggesting that there were multiple sources of this gene and/or that it undergoes transport by the water column. Sediment-associated cry1Ab gene from Bt corn tended to decrease with distance from the Bt cornfield. Sediment concentrations of the cry1Ab gene were significantly correlated with those of the cry1Ab gene in surface water (R=0.83;P=0.04). The data indicate that DNA from Bt corn and Bt were persistent in aquatic environments and were detected in rivers draining farming areas.

http://www.ncbi.nlm.nih.gov/pubmed/16499967

Serratos-Hernández, J-A.; Gómez-Olivares, J-L.; Salinas-Arreortua, N.; Buendía-Rodríguez, E.; Islas-Gutiérrez, F.; de-Ita, A. 2007. Transgenic proteins in maize in the Soil Conservation area of Federal District, Mexico. *Frontiers in Ecology and the Environment*, 5: 247-252.

In 2003, the environmental authorities of the Federal District of Mexico declared that genetically modified organisms were incompatible with ecological agriculture practices established in rural areas south of Mexico City. To ensure compliance with official standards and organic agriculture policies, steps were taken to implement an early warning system for the detection of genetically modified maize in farmers' fields. In our sampling efforts, which were conducted in 2003, transgenic proteins expressed in maize were found in two (0.96%) of 208 samples from farmers' fields, located in two (8%) of 25 sampled communities. Mexico imports a substantial amount of maize from the US, and due to formal and informal seed networks among rural farmers, there are many potential routes of entrance for transgenic maize into food and feed webs. To sustain agroecological practices, preserve organic agriculture, and conserve maize landraces in the Soil Conservation area of the Mexican Federal District, environmental authorities will need to maintain and update ecological policies such as the "green seal" for organic agriculture, apply alternative technologies such as biofertilizers to enhance plant nutrition, and develop sustainable maize agriculture with the implementation of profitable intercropping systems.

http://www.esajournals.org/doi/abs/10.1890/1540-9295%282007%295%5B247%3ATPIMIT%5D2.0.CO%3B2?journalCode=fron

Icoz, I.; Saxena, D.; Andow, D.; Zwahlen, C.; Stotzky, G. 2008. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing cry proteins from *Bacillus thuringiensis*. *J Environ Qual*, 37(2):647-62.

Transgenic Bt crops produce insecticidal Cry proteins that are released to soil in plant residues, root exudates, and pollen and that may affect soil microorganisms. As a continuation of studies in the laboratory and a plant-growth room, a field study was conducted at the Rosemount Experiment Station of the University of Minnesota. Three Bt corn varieties that express the Cry1Ab protein, which is toxic to the European corn borer (Ostrinia nubilalis Hübner), and one Bt corn variety that expresses the Cry3Bb1 protein, which is toxic to the corn rootworm complex (Diabrotica spp.), and their near-isogenic non-Bt varieties were evaluated for their effects on microbial diversity by classical dilution plating and molecular (polymerase chain reaction-denaturing gradient gel electrophoresis) techniques and for the activities of some enzymes (arylsulfatases, acid and alkaline phosphatases, dehydrogenases, and proteases) involved in the degradation of plant biomass. After 4 consecutive years of corn cultivation (2003-2006), there were, in general, no consistent statistically significant differences in the numbers of different groups of microorganisms, the activities of the enzymes, and the pH between soils planted with Bt and non-Bt corn. Numbers and types of microorganisms and enzyme activities differed with season and with the varieties of corn, but these differences were not related to the presence of the Cry proteins in soil. The Cry1Ab protein of Bt corn (events Bt11 and MON810) was detected in most soils during the 4 yr, whereas the Cry3Bb1 protein was not detected in soils of Bt corn (event MON863) expressing the cry3Bb1 gene.

http://www.ncbi.nlm.nih.gov/pubmed/18396552

Tank, J.; Rosi-Marshall, E.; Royer, T.; Whiles, M.; Griffiths, N.; Frauendorf, T.; Treering, D. 2010. Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. *PNAS*.

Widespread planting of maize throughout the agricultural Midwest may result in detritus entering adjacent stream ecosystems, and 63% of the 2009 US maize crop was genetically modified to express insecticidal Cry proteins derived from *Bacillus thuringiensis*. Six months after harvest, we conducted a synoptic survey of 217 stream sites in Indiana to determine the extent of maize detritus and presence of Cry1Ab protein in the stream network. We found that 86% of stream sites contained maize leaves, cobs, husks, and/or stalks in the active stream channel. We also detected Cry1Ab protein in stream-channel maize at 13% of sites and in the water column at 23% of sites. We found that 82% of stream sites were adjacent to maize fields, and Geographical Information

Systems analyses indicated that 100% of sites containing Cry1Ab-positive detritus in the active stream channel had maize planted within 500 m during the previous crop year. Maize detritus likely enters streams throughout the Corn Belt; using US Department of Agriculture land cover data, we estimate that 91% of the 256,446 km of streams/rivers in Iowa, Illinois, and Indiana are located within 500 m of a maize field. Maize detritus is common in low-gradient stream channels in northwestern Indiana, and Cry1Ab proteins persist in maize leaves and can be measured in the water column even 6 mo after harvest. Hence, maize detritus, and associated Cry1Ab proteins, are widely distributed and persistent in the headwater streams of a Corn Belt landscape.

Artigo completo em http://www.pnas.org/content/107/41/17645.full

Fu, Q.; Chen, S.; Hu, H.; Li, Z.; Han, X.; Wang, P. 2011. Distribution of Bt protein in transgenic cotton soils. [Article in Chinese]. *Ying Yong Sheng Tai Xue Bao*, 22(6):1493-8.

A pot experiment with red soil, yellow brown soil, and yellow cinnamon soil was conducted to detect the Bt protein content in rhizosphere and non-rhizosphere soils at different growth stages of transgenic Bt cotton and common cotton by using enzyme linked immunosorbent assay (ELISA). With the planting of transgenic Bt cotton, the Bt protein content in rhizosphere soil was significantly higher than that in non-rhizosphere soil; while in common cotton soils, there was no significant difference in the Bt protein content between rhizosphere soil and non-rhizosphere soil. At bud stage of transgenic Bt cotton, the Bt protein content in rhizosphere soil was in the order of yellow cinnamon soil > yellow brown soil > red soil, being 144% 121%, and 238% of that in common cotton rhizosphere soil; at florescence stage of transgenic Bt cotton, the Bt protein content in rhizosphere soil was in the order of yellow brown soil > yellow cinnamon soil > red soil, being 156% , 116% , and 197% of that in common cotton rhizosphere soil, respectively. Regardless of planting Bt cotton or common cotton, the Bt protein content in rhizosphere and non-rhizosphere soils had an initial increase with the growth of cotton, peaked at florescence stage, and then decreased. Throughout the whole cotton growth period, the Bt protein content in transgenic Bt cotton rhizosphere soil was higher than that in Bt cotton non-rhizosphere soil, and also, higher than that in common cotton rhizosphere soil, indicating that transgenic Bt cotton could release its Bt protein to rhizosphere soil.

http://www.ncbi.nlm.nih.gov/pubmed/21941750

1.3.3 Desequilíbrios observados em comunidades do solo afetadas pela pressão de cultivos Bt

Entre os riscos associados à presença das toxinas Cry no solo – por períodos de tempo significativos e em formas biologicamente ativas – está sua ação desequilibradora sobre composições populacionais das comunidades ali estabelecidas.

Trata-se de área de pesquisa em pleno desenvolvimento[44]. Aqui o estudo de impactos ambientais decorrentes do cultivo de plantas Bt oferece artigos científicos frequentemente divergentes. Boa parte

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[44] Nesses últimos anos a sociomicrobiologia tende a se fortalecer como nova abordagem das relações estreitas existentes nessa biota do solo.

desses estudos aponta para distúrbios aparentemente pouco significativos, ainda que com registros de alterações e danos sobre grupos funcionais relevantes, de modo que as consequências de longo prazo (sobre a saúde do solo) ainda estão longe de ser conhecidas.

Como referido em capítulos anteriores, também nesse caso as condições ambientais e de manejo (tipo de solo, de clima e de manejo praticado, tal como a presença ou não de cobertura vegetal) influenciam os resultados das pesquisas, inviabilizando conclusões genéricas.

Em consequência, as informações apontam a necessidade de estudos cuidadosos, com base em protocolos adequados, caso a caso. Justifica-se, dessa maneira, o estabelecimento de estudos de longo prazo, apoiados em cenários reais, representativos dos agroecossistemas e biomas potencialmente afetados, sem os quais as avaliações do risco permanecerão insuficientes e inadequadas.

Sun, C.; Chen, L.; Wu, Z.; Zhang, Y.; Zhang, L. 2003. Effect of transgenic Bt rice planting on soil enzyme activities. [Article in Chinese]. *Ying Yong Sgeng Tai Xue Bao*, 14: 2261-2264.

A pot experiment was conducted with silty loam Agrodolf as test soil and with transgenic Bt rice and non-Bt rice as test crops to study the effect of transgenic Bt rice planting on soil urease, phophatase, arylsulfatase, invertase, and dehydrogenase activities. The results showed that Bt toxin could be introduced into soil through root exudates of transgenic Bt rice, and its survival amount in soil varied with time. Compared with non-Bt rice treatment, transgenic Bt rice treatment had a significant decrease (2.47%) of soil urease activity and a significant increase (8.91%) of soil acid phosphatase activity, but no significant change in soil arylsulfatase, invertase, and dehydrogenase activities at the 15th day of emergence. At the 30th day of emergence, the transgenic Bt rice treatment still had a significant decrease of soil urease activity (16.36%) and a significant increase of acid phosphatase activity (35.69%), and no change in invertase activity. It also had significant increase in soil arylsulfatase (19.70%) and dehydrogenase activities (16.83%).

http://www.ncbi.nlm.nih.gov/pubmed/15031930

REV – Bruinsma, M.; Kowalchuk, G.; Veen, J. 2003. Effects of genetically modified plants on microbial communities and processes in soil. *Biology and Fertility of Soils*, 37: 329-337.

The development and use of genetically modified plants (GMPs) has been a topic of considerable public debate in recent years. GMPs hold great promise for improving agricultural output, but the potential for unwanted effects of GMP use is still not fully understood. The majority of studies addressing potential risks of GMP cultivation have addressed only aboveground effects. However, recent methodological advances in soil microbial ecology have allowed research focus to move underground to try to gain knowledge of GMP-driven effects on the microbial communities and processes in soil that are essential to key terrestrial ecosystem functions. This review gives an overview of the research performed to date on this timely topic, highlighting a number of case studies. Although such research has advanced our understanding of this topic, a number of knowledge gaps still prevent full interpretation of results, as highlighted by the failure of most studies to assign a definitively negative, positive or neutral effect to GMP introduction. Based upon our accumulating, yet incomplete, understanding of soil microbes and processes, we propose a synthesis for the case-by-case study of GMP effects, incorporating assessment of the potential plant/ecosystem interactions, accessible and relevant indicators, and tests for unforeseen effects.

http://link.springer.com/article/10.1007%2Fs00374-003-0613-6

Wu, W-X.; Ye, Q-F.; Min, H. 2004. Effect of straws from Bt-transgenic rice on selected biological activities in water-flooded soil. *European Journal of Soil Biology*, 40 (1): 15-22.

The biochemical properties of soil have often been described as early and sensitive indicators of ecological changes in both natural soil and agroecosystem. In the current study, the impacts of the amendment of Bt-transgenic rice (KMD) straw on biological activities in water-flooded soil were investigated under laboratory conditions and compared with non-transgenic rice (Xiushui 11) straw. The results showed that there were some differences in protease, neutral phosphatase and cellulase activities between soil amended with Bt-transgenic rice straw and non-transgenic rice straw at the early stage of incubation, and none of these differences were persistent. However, differences in dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration between soil supplemented with Bt-transgenic rice straw and non-transgenic rice straw were persistent over the course of incubation. Dehydrogenase activity, methanogenesis and anaerobic respiration were considerably lower from sample days 7 to 56, but higher after day 56 in soil amended with Bt-transgenic rice straw. In comparison, the H_2-production in soil containing Bt-transgenic rice straw was significantly lower after day 56. The results demonstrated that the amendment of the Bt-transgenic rice straw altered some important biological properties in water-flooded soil, indicating a shift in microbial populations or a change in the metabolic abilities of the microbial community as a result of substrate availability in soil.

http://www.sciencedirect.com/science/article/pii/S1164556304000044

REV – Dunfield, K.; Germida, J. 2004. Impact of genetically modified crops on soil and plant-associated microbial communities. *Journal of Environmental Quality*, 33 (3), 806-815.

Transgenic or genetically modified plants possess novel genes that impart beneficial characteristics such as herbicide resistance. One of the least understood areas in the environmental risk assessment of genetically modified crops is their impact on soil- and plant-associated microbial communities. The potential for interaction between transgenic plants and plant residues and the soil microbial community is not well understood. The recognition that these interactions could change microbial biodiversity and affect ecosystem functioning has initiated a limited number of studies in the area. At this time, studies have shown the possibility that transgenes can be transferred to native soil microorganisms through horizontal gene transfer, although there is not evidence of this occurring in the soil. Furthermore, novel proteins have been shown to be released from transgenic plants into the soil ecosystem, and their presence can influence the biodiversity of the microbial community by selectively stimulating the growth of organisms that can use them. Microbial diversity can be altered when associated with transgenic plants; however, these effects are both variable and transient. Soil- and plant-associated microbial communities are influenced not only by plant species and transgene insertion but also by environmental factors such as field site and sampling date. Minor alterations in the diversity of the microbial community could affect soil health and ecosystem functioning, and therefore, the impact that plant variety may have on the dynamics of the rhizosphere microbial populations and in turn plant growth and health and ecosystem sustainability, requires further study.

https://dl.sciencesocieties.org/publications/jeq/abstracts/33/3/0806

Castaldini, M.; Turrini, A.; Sbrana, C.; Benedetti, A.; Marchionni, M.; Mocali, S.; Fabiani, A.; Landi, S.; Santomassimo, F.; Pietrangeli, B.; Nuti, M.; Miclaus, N.; Giovannetti, M. 2005. Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. *Applied and Environmental Microbiology*, 71(11): 6719-29.

A polyphasic approach has been developed to gain knowledge of suitable key indicators for the evaluation of environmental impact of genetically modified Bt 11 and Bt 176 corn lines on soil ecosystems. We assessed the effects of Bt corn (which constitutively expresses the insecticidal toxin from *Bacillus thuringiensis*, encoded by the truncated *Cry1Ab* gene) and non-Bt corn plants and their residues on rhizospheric and bulk soil eubacterial communities by means of denaturing gradient gel electrophoresis analyses of 16S rRNA genes, on the nontarget mycorrhizal symbiont *Glomus mosseae*, and on soil respiration. Microcosm experiments showed differences in rhizospheric eubacterial communities associated with the three corn lines and a significantly lower level of mycorrhizal colonization in Bt 176 corn roots. In greenhouse experiments, differences between Bt and non-Bt corn plants were detected in rhizospheric eubacterial communities (both total and active), in culturable rhizospheric heterotrophic bacteria, and in mycorrhizal colonization. Plant residues of transgenic plants, plowed under at harvest and kept mixed with soil for up to 4 months, affected soil respiration, bacterial communities, and mycorrhizal establishment by indigenous endophytes. The multimodal approach utilized in our work may be applied in long-term field studies aimed at monitoring the real hazard of genetically modified crops and their residues on nontarget soil microbial communities.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1287690/

Mulder, C.; Wouterse, M.; Raubuch, M.; Roelofs, W.; Rutgers, M. 2006. Can transgenic maize affect soil microbial communities? *PLOS Computational Biology*, 2(9): e128. DOI: 10.1371/journal.pcbi.0020128.

The aim of the experiment was to determine if temporal variations of belowground activity reflect the influence of the Cry1Ab protein from transgenic maize on soil bacteria and, hence, on a regulatory change of the microbial community (ability to metabolize sources belonging to different chemical guilds) and/or a change in numerical abundance of their cells. Litter placement is known for its strong influence on the soil decomposer communities. The effects of the addition of crop residues on respiration and catabolic activities of the bacterial community were examined in microcosm experiments. Four cultivars of *Zea mays* L. of two different isolines (each one including the conventional crop and its *Bacillus thuringiensis* cultivar) and one control of bulk soil were included in the experimental design. The growth models suggest a dichotomy between soils amended with either conventional or transgenic maize residues. The Cry1Ab protein appeared to influence the composition of the microbial community. The highly enhanced soil respiration observed during the first 72 h after the addition of *Bt*-maize residues can be interpreted as being related to the presence of the transgenic crop residues. This result was confirmed by agar plate counting, as the averages of the colony-forming units of soils in conventional treatments were about one-third of those treated with transgenic straw. Furthermore, the addition of *Bt*-maize appeared to induce increased microbial consumption of carbohydrates in BIOLOG EcoPlates. Three weeks after the addition of maize residues to the soils, no differences between the consumption rate of specific chemical guilds by bacteria in soils amended with transgenic maize and bacteria in soils amended with conventional maize were detectable. Reaped crop residues, comparable to post-harvest maize straw (a common practice in current agriculture), rapidly influence the soil bacterial cells at a functional level. Overall, these data support the existence of short *Bt*-induced ecological shifts in the microbial communities of croplands' soils.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1584322/

Sun, C.; Chen, L.; Wu, Z.; Zhou, L.; Shimizu, H. 2007. Soil persistence of *Bacillus thuringiensis* (Bt) toxin from transgenic Bt cotton tissues and its effect on soil enzyme activities. *Biology and Fertility of Soils*, 43 (5): 617-620.

A silty loam soil was incubated with the leaves and stems of two transgenic Bacillus thuringiensis (Bt) cotton varieties and nontransgenic Bt cotton to study the soil persistence of the Bt toxin from the decomposing transgenic Bt cotton tissues and its effect on soil enzyme activities. The results showed that after Bt cotton tissue amendment, Bt toxin was introduced into soil upon decomposition; about 50% of the introduced Bt toxin persisted in soil for at least 56 days. No Bt toxin was detected in the nontransgenic Bt cotton-amended soil; the amount of Bt toxin was the highest in the soil treated with the residue with the higher Bt toxin content. Activities of soil urease, acid phosphomonoesterase, invertase, and cellulase were stimulated by the addition of Bt cotton tissues, whereas activity of soil arylsulfatase was inhibited. Probably cotton tissue stimulated microbial activity in soil, and as a consequence, enzyme activities of soil were generally increased. This effect can mask any negative effect of the Bt toxin on microbial activity and thus on enzyme activities.

http://link.springer.com/article/10.1007%2Fs00374-006-0158-6

Sarkar, B.; Patra, A.; Purakayastha, T. 2008. Transgenic Bt-Cotton affects enzyme activity and nutrient availability in a sub-tropical inceptisol. *J Agronomy & Crop Science*, ISSN 0931-2250.

We investigated the dynamics of N and P availability in the rhizosphere of Bt and non-Bt cotton crops during their growth. In a net-house pot culture experiment at the Indian Agricultural Research Institute, New Delhi, Bt-cotton (cv. MRC-6301Bt) and its non-transgenic near-isoline (MRC-6301) were grown on a sandy loam soil until maturity. A control (no-crop) treatment was also included. Rhizosphere soil and root samples were collected at 60, 90, and 120 days after sowing (DAS). Soil samples were analysed for dehydrogenase activity, soil respiration, mineral-N and Olsen-P. Results have revealed a significant reduction in dehydrogenase activity ()17 %) and soil respiration ()3.5 %) in the rhizosphere of Bt-cotton over non-Bt isoline. Total mineral-N (NH4+-N + NO_3 -N) in soil was reduced by 14 %, whereas Olsen-P was increased by 8 % because of Bt-cotton. Root biomass yields were not diferente ($P > 0.05$), but root volume was significantly higher in Bt than non-Bt isoline. Time of sampling strongly ($P < 0.05$) affected the above parameters, showing their highest values at 60 or 90 DAS. A significant interactive effect of sampling time and treatments was also indicated. Our results suggest that Bt-cotton may constrain the availability of N, but enhances P-availability in these soils.

Artigo completo disponível em http://stopogm.net/files/TropicalInceptisol.pdf

Cheeke, T.; Pace, B.; Rosenstiel, T.; Cruzan, M. 2011. The influence of fertilizer level and spore density on arbuscular mycorrhizal colonization of transgenic Bt 11 maize (Zea mays) in experimental microcosms. *FEMS Microbiol Ecol*, 75(2): 304-312.

Crop plants genetically modified for the expression of Bacillus thuringiensis (Bt) insecticidal toxins have broad appeal for reducing insect damage in agricultural systems, yet questions remain about the impact of Bt plants on symbiotic soil organisms. Here, arbuscular mycorrhizal fungal (AMF) colonization of transgenic maize isoline Bt 11 (expressing Cry1Ab) and its non-Bt parental line (Providence) was evaluated under different fertilizer level and spore density scenarios. In a three-way factorial design, Bt 11 and non-Bt maize were inoculated with 0, 40, or 80 spores of Glomus mosseae and treated weekly with ‘No’ (0 g L(-1)), ‘Low’ (0.23 g L(-1)), or ‘High’ (1.87 g L(-1)) levels of a complete fertilizer and grown for 60 days in a greenhouse. While no difference in AMF colonization was detected between the Bt 11 and Providence maize cultivars in the lower spore/higher fertilizer treatments, microcosm experiments demonstrated a significant reduction in AMF colonization in Bt 11 maize roots in the 80 spore treatments when fertilizer was limited. These results confirm previous work indicating an altered relationship between this Bt 11 maize isoline and AMF and demonstrate that the magnitude of this response is strongly dependent on both nutrient supply and AMF spore inoculation level.

http://www.ncbi.nlm.nih.gov/pubmed/21198682

Chen, Z.; Chen, L.; Zhang, Y.; Wu, Z. 2011. Microbial properties, enzyme activities and the persistence of exogenous proteins in soil under consecutive cultivation of transgenic cottons (*Gossypium hirsutum L.). Plant Soil and Environment*, UZEI 57 (2): 67-74.

One *Bacillus thuringiensis* (*Bt*) and two stacked *Bt* and cowpea trypsin inhibitor (*Bt* + *CpTI*) cottons and their non-transgenic isolines were consecutively cultivated to investigate the soil persistence of *Cry1Ac* and *CpTI* proteins and their effects on microbial properties and enzyme activities involving C, N, P, and S cycling in soil. Results showed that there were the persistence of *Cry1Ac* and *CpTI* proteins in soil under 4-year consecutive cultivation of transgenic cottons. *Cry1Ac* proteins varied from 6.75 ng/g to 12.01 ng/g and *CpTI* proteins varied from 30.65 to 43.60 ng/g. However, neither of these two proteins was detected in soil under non-transgenic cottons. Soil microbial biomass carbon, microbial activities, and soil enzyme activities (except urease and phosphodiesterase) significantly decreased in soil under transgenic cottons. Correlation analysis showed that most of microbial properties and enzyme activities in soil had a negative relationship with *Cry1Ac* content, while most of them had a positive relationship with *CpTI* content. Our data indicate that consecutive cultivation by genetically modified cottons with *Bt* and *CpTI* genes can result in persistence of *Cry1Ac* and *CpTI* proteins and negatively affect soil microbial and biochemical properties.

Artigo completo disponível em http://www.agriculturejournals.cz/publicFiles/35214.pdf

Zhou, X.; Liu, N.; Zhao, M.; Li, H.; Zhou, L.; Tang, Z.; Cao, F.; Li, W. 2011. Advances in effects of insecticidal crystal proteins released from transgenic Bt crops on soil ecology. [Article in Chinese]. *Yi Chuan*, 33(5):443-8.

With the large scale cultivation of transgenic crops expressing Bacillus thuringiensis (Bt) insecticidal crystal proteins in the world, the problem of environmental safety caused by these Bt crops has received extensive attention. These insecticidal crystal proteins can be released into the soil continuously in the growing period of Bt plants. If their accumulation of the insecticidal crystal proteins exceeds consumption by insect larvae and degradation by the environmental factors, these insecticidal crystal proteins could constitute a hazard to non-target insects and soil microbiota. There are three main ways to release insecticidal crystal proteins into soil for Bt plants: root exudates, pollen falling, and crop reside returning. The Bt insecticidal crystal proteins released into soil can be adsorbed rapidly by active soil particles and the absorption equilibrium attained within 1-3 h. The adsorption protects Bt insecticidal crystal proteins against soil microbial degradation or enzyme degradation, which leads to remarkable prolong of the persistence of insecticidal activity. The change of soil microorganism species is an important index for evaluating the effect of Bt plants on soil ecology. The research showed that these insecticidal crystal proteins released by the Bt plant root exudates or Bt organism had no toxicity to the soil earthworms, nematodes, protozoa, bacteria and fungi; however, it could reduce the mycelium length of the arbuscular mycorrhizal fungi (AMF) and restrain AMF to form invasion unit. The influencing degree of Bt protein on soil enzyme activity varied with the releasing modes or growth period of Bt crops. Bt Cry1Ab protein can be taken up from soil by parts of following crops; however, different results were obtained with different commercial kits. To better understand the soil ecological evaluation about the insecticidal crystal proteins released from transgenic Bt crops, this review provides a comprehensive overview about the release, adsorption and residue of Bt insecticidal crystal proteins in soil, as well as their effects on soil protozoa, soil microorganism, soil enzyme activity and following crops.

http://www.ncbi.nlm.nih.gov/pubmed/21586391

Tarafdar, J.; Rathore, I.; Shiva, V. 2012. Effect of Bt- transgenic cotton on soil biological health. *Applied Biological Research*, 14 (1): 15-23.

Bt cotton are plants that have been genetically modified to express the insecticidal proteins *Cry 1 Ac* from subspecies of the bacterium, *Bacillus thuringiensis* israelensis (Bt), to control bollworm pest that feed on cotton. There is a persistent environmental concern that transgenic Bt-crops carry genes that have indirect undesirable effect to natural and agro–ecosystem function. We investigated the effect of Bt-cotton (with *Cry 1 Ac* gene) on several microbial and biochemical indicators in fields under subhumid tropical condition. Twenty five fields were selected in the Vidarbha region, India, where Bt-cotton has been growing at least three consecutive years and side by side field of non-transgenic cotton is growing under clay to clay loam soil. Soil from a control (no-crop) treatment was also included from each area to compare the extent of adverse effect of Bt, if any. Samples were analyzed for actinobacteria, fungi and nitrifiers population, biomass carbon (MBC), biomass nitrogen (MBN), biomass phosphorus (MBP) and soil enzyme activities. The result revealed a significant decline in actinobacteria (17%), bacterial (14%) count as well as acid phosphatases (27%), phytase (18%), nitrogenase (23%) and dehydrogenase (12%) activities in Bt cotton compared with non-Bt cotton fields. Fungal and nitrifiers counts and esterase and alkaline phosphatase activities were not affected by the introduction of Bt-cotton in fields. However, significant decline between 8 and 9% in MBC and MBN was also noticed.

Artigo completo disponível em http://afsafrica.org/wp-content/uploads/2014/08/ABR-14-_1_-02-00-Bt-cotton-Dr-I-Rathore.pdf

Cheeke, T.; Rosenstiel, T.; Cruzan, M. 2012. Evidence of reduced arbuscular mycorrhizal fungal colonization in multiple lines of Bt maize. *American Journal of Botany*, 99(4): 700707.

Premise of the Study: Insect-resistant Bacillus thuringiensis (Bt) maize is widely cultivated, yet few studies have examined the interaction of symbiotic arbuscular mycorrhizal fungi (AMF) with different lines of Bt maize. As obligate symbionts, AMF may be sensitive to genetic changes within a plant host. Previous evaluations of the impact of Bt crops on AMF have been inconsistent, and because most studies were conducted under disparate experimental conditions, the results are difficult to compare.
Methods: We evaluate AMF colonization in nine Bt maize lines, differing in number and type of engineered trait, and five corresponding near-isogenic parental (P) base hybrids in greenhouse microcosms. Plants were grown in 50% local agricultural soil with low levels of fertilization, and AMF colonization was evaluated at 60 and 100 d. Nontarget effects of Bt cultivation on AMF colonization were tested in a subsequently planted crop, Glycine max, which was seeded into soil that had been preconditioned for 60 d with Bt or P maize.
Key Results: We found that Bt maize had lower levels of AMF colonization in their roots than did the non-Bt parental lines. However, reductions in AMF colonization were not related to the expression of a particular Bt protein. There was no difference in AMF colonization in G. max grown in the Bt- or P-preconditioned soil.
Conclusions: These findings are the first demonstration of a reduction in AMF colonization in multiple Bt maize lines grown under the same experimental conditions and contribute to the growing body of knowledge examining the unanticipated effects of Bt crop cultivation on nontarget soil organisms

http://www.ncbi.nlm.nih.gov/pubmed/22473978

2 Riscos para o meio ambiente associados ao uso de plantas tolerantes a herbicidas (tecnologia TH)

As chamadas plantas tolerantes a herbicidas foram geneticamente modificadas com vistas a facilitar o controle de plantas concorrentes, em disputas pela ocupação do solo, em áreas de lavoura. Em termos práticos isso foi obtido mediante a inserção de transgenes que permitiram às PGMs-TH absorver e metabolizar determinados herbicidas, sem que isso lhes provocasse danos letais. Assim, banhos de agrotóxicos aplicados em qualquer momento e tantas vezes quanto desejado, sobre toda a área de cultivo, eliminariam o restante da cobertura vegetal, reduzindo a concorrência da cultura principal, por luz, água e nutrientes, simplificando radicalmente o manejo e o controle de "plantas invasoras".

Os proponentes da tecnologia afirmavam que, com os monocultivos TH, as plantas ruderais, indesejáveis, seriam controladas, e que isso traria escassos efeitos colaterais. De fato, nos primeiros anos assim pareceu ocorrer. Entretanto, como desdobramentos de médio prazo, foram constatadas profundas modificações nos ecossistemas. Estudos acumularam evidências de impactos para muito além de questões relacionadas ao manejo das chamadas plantas "daninhas". Reduziu-se e homogeneizou-se o banco de sementes no solo, expandiu-se o uso dos herbicidas associados e passaram a ser observadas alterações substanciais nas comunidades de organismos estabelecidos no solo, no subsolo e nas águas dos agroecossistemas envolvidos.

Os impactos ambientais não apenas determinaram o surgimento de plantas tolerantes (demandando ampliação no uso e alteração nos princípios ativos, que como regra "evoluíram" para formulações mais danosas à saúde e ao ambiente) como trouxeram implicações diversas, afetando negativamente comunidades de ambientes terrestres, aquáticos e do subsolo das regiões cultivadas com plantas TH.

Por esses motivos, as avaliações de risco de plantas TH devem levar em conta os herbicidas a elas associados.

Os artigos a seguir desenvolvem essa perspectiva.

2.1 Impactos negativos específicos dos principais herbicidas associados ao cultivo de PGMs TH

Neste item são avaliados três dos principais grupos de herbicidas para os quais plantas TH foram desenvolvidas (glifosato, glufosinato de amônio e 2,4-D). Maior destaque é oferecido à família dos herbicidas à base de glifosato, tanto por seu uso dominante como pelo acúmulo de informações disponíveis. Importante mencionar que, apesar de farta documentação comprovando seus impactos deletérios para a saúde humana, animal e ambiental, ainda se trata de agroquímico classificado como de baixa toxicidade. Recentemente (após a conclusão deste livro) o IARC (Agência Internacional de Pesquisa sobre o Câncer) o classificou como possivelmente cancerígeno, o que deflagrou processos de reavaliação que podem determinar sua reclassificação ou mesmo proibição de uso, no Brasil.

2.1.1 Glifosato e fórmulas comerciais à base de glifosato

Contrariamente à propaganda historicamente desenvolvida pelo setor dos agrotóxicos[45] e outros defensores da tecnologia, os herbicidas à base de glifosato não são biodegradáveis nem de baixa toxicidade para a saúde humana[46] e o meio ambiente.

Perturbações endócrinas, genotoxicidade, mutagenicidade e até mudanças comportamentais em populações de organismos em contato forçado com esse herbicida estão relatadas em dezenas de estudos constantes na literatura científica. Por meio da sua ação sistêmica, as enormes quantidades de glifosato despejadas em dezenas de milhões

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[45] A empresa inicialmente responsável pelo herbicida da marca Roundup, à base de glifosato, foi efetivamente condenada duas vezes pela justiça por propaganda enganosa, uma vez na França e outra vez nos EUA.

[46] Nos itens 2.1.1 e 2.2.2 da Parte 4 existem artigos adicionais tratando de riscos para a saúde, associados ao uso de herbicidas à base de glifosato.

de hectares ao redor do mundo têm provocado degradação significativa de ambientes naturais e seminaturais – e da biocenose que os compõe. Os meios aquáticos e semiaquáticos estão sendo impactados de forma mais aguda e dramática por esses herbicidas.

Alguns dos estudos relatados a seguir acusam ecotoxicidade em animais modelo (tais como ouriços, determinados anfíbios ou aves) e mamíferos, cobrindo também o campo dos riscos para a saúde humana.

Danos observados sobre a biota do solo e micro-organismos em geral sugerem indicativos de problemas agronômicos, cujos impactos socioeconômicos tendem a ser alarmantes. No item 3.2 da Parte 2 existem informações adicionais sobre a temática trabalhada nos artigos apresentados a seguir.

Santillo, D.; Brown, P.; Leslie, D. 1989. Response of songbirds to glyphosate-induced habitat changes on clearcuts. *Journal of Wildlife Management*. 53: 64–71.

We examined *breeding* bird populations and habitats on glyphosate (nitrogen-phosphonomethyl glycine) (Roundup, Monsanto, St. Louis, Mo.)-treated and untreated clearcuts in north-central Maine. Treatment of clearcuts with glyphosate herbicide reduced the complexity of vegetation through *3 years* post-treatment compared to untreated clearcuts. Total *numbers* of birds, common yellowthroats (Geothlypis trichas), Lincoln's sparrows (Melospiza lincolnii), and alder flycatchers (Empidonax alnorum) were less abundant ($P < 0.05$) on treated clearcuts than on untreated clearcuts. Songbird densities were correlated with habitat complexity, especially hardwood regeneration, foliage height diversity (FHD), and vegetation height. Leaving untreated patches of vegetation and staggering herbicide treatments on large clearcuts will maintain bird populations similar to those of untreated clearcuts.

https://eurekamag.com/research/007/751/007751516.php

Springett, J.; Gray, R. 1992. Effect of repeated low doses of biocides on the earthworm Aporrectodea caliginosa in laboratory culture. *Soil Biology and Biochemistry,* 24: 1739–1744.

The growth rates of *Aporrectodea caliginosa* (Savigny) were measured over a 100-day period in soil in culture chambers which were treated with common biocides singly and in combination. The biocides used were: the fungicide Captan, the herbicide, Glyphosate and the insecticide, Azinphos-methyl. The biocides were applied at intervals of 14 days and each treatment was replicated six times. The results are variable, all biocides depressed growth when applied alone but some combinations reduced the effect of other biocides. Azinphos-methyl and Glyphosate applied alone, reduced growth the most over the 100 days and at all rates of application. Azinphos-methyl applied at the highest rate killed worms. Captan applied alone had the least effect on growth and mortality. In combination, Glyphosate and Captan had a lesser effect than Glyphosate alone. Azinphos-methyl and Captan had less effect than Azinphos-methyl alone. After 100 days the combination of all three biocides reduced growth to the same degree as Glyphosate alone.

http://www.sciencedirect.com/science/article/pii/0038071792901806

Tate, T.; Spurlock, J.; Christian, F. 1997. Effect of glyphosate on the development of *Pseudosuccinea columella* snails. *Archives of Environmental Contamination and Toxicology,* 33: 286-9.

Glyphosate (Roundup) is one of the most commonly used broad-spectrumherbicides with little to no hazard to animals, man, or the environment. Due to its widespread use, there is continuous contamination of the environment in both soil and water with this herbicide. There is a paucity of long-term exposure studies with sublethal concentrationsof glyphosate on aquatic snails. This study was developed to determine the effects of sublethal concentrations of glyphosate on development and survival of Pseudosuccinea columella (intermediate snail host of Fasciola hepatica). This was assessed by continuously exposing three successive generations of snails to varying concentrations (0.1-10mg/L) of glyphosate. Glyphosate had little effect on the first- and second-generation snails. However, third-generation snail embryos exposed to 1.0 mg/L glyphosate developed much faster than other embryos exposed to 0.1 mg/L, 10 mg/L, and 0 mg/L (control). Hatching was inhibited at 10 mg/L and inhibited slightly at 0.1 mg/L. The egg-laying capacity was increased in snails exposed to 0.1 and 10 mg/L. Abnormalities and polyembryony were observed in snails exposed to 0.1 and 10 mg/L. These results indicate that glyphosate does affect snail reproduction and development. This, in turn, could possibly have an effect on the population dynamics of F. hepatica, which could result in increased infections in animals, including man.

http://link.springer.com/article/10.1007%2Fs002449900255

Newmaster, S.; Bell, F.; Vitt, D. 1999. The effects of glyphosate and triclopyr on common bryophytes and lichens in northwestern Ontario. *Canadian Journal of Forest Research*, 29, 1101-1111.

The effects of two silvicultural herbicides (Vision®, Release®) on bryophytes and lichens were studied in a harvested boreal mixedwood ecosystem. A completely randomized design with 115 plots of 1 m^2 allowed direct comparison between herbicides and their effects on community dynamics. Regression models were used to analyze the relationship between herbicide application rates (0.71-6.72 kg active ingredient/ha) and changes in bryophyte and lichen abundance and species richness for 2 years following herbicide application. Results showed that bryophyte and lichen abundance and species richness decreased after herbicide treatments. In general, herbicide applications reduced the diversity of forest mesophytes and weedy colonizers to an ecosystem with only a few species of colonizers. A combination of clustering techniques and ANOVA were used to divide bryophytes and lichens into three ecologically defined response groups: herbicide-tolerant colonizers, semi tolerant long-term stayers from dry open forest, and sensitive forest mesophytes.

http://www.nrcresearchpress.com/doi/abs/10.1139/x99-083#.VNTl9Sd4tXg

Relyea, R. 2005. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications*, 15, 618-627.

Pesticides constitute a major anthropogenic addition to natural communities. In aquatic communities, a great majority of pesticide impacts are determined from singlespecies experiments conducted under laboratory conditions. Although this is an essential protocol to rapidly identify the direct impacts of pesticides on organisms, it prevents na assessment of direct and indirect pesticide effects on organisms embedded in their natural ecological contexts. In this study, I examined the impact of four globally common pesticides (two insecticides, carbaryl [Sevin] and malathion; two herbicides, glyphosate [Roundup] and 2,4-D) on the biodiversity of aquatic communities containing algae and 25 species of animals. Species richness was reduced by 15% with Sevin, 30% with malathion, and 22% with Roundup, whereas 2,4-D had no effect. Both insecticides reduced zooplankton diversity by eliminating cladocerans but not copepods (the latter increased in abundance). The insecticides also reduced the diversity and biomass of predatory insects and had an apparent indirect positive effect on several species of tadpoles, but had no effect on snails. The two herbicides had no effects on zooplankton, insect predators, or snails. Moreover, the herbicide 2,4-D had no effect on tadpoles. However, Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles. This study represents one of the most extensive experimental investigations of pesticide effects on aquatic communities and offers a comprehensive perspective on the impacts of pesticides when nontarget organisms are examined under ecologically relevant conditions.

Artigo completo disponível em https://www.whyy.org/91FM/ybyg/relyea2005.pdf

Relyea, R. 2005. The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecological Applications*, 15 (4): 1118-1124.

The global decline in amphibian diversity has become an international environmental problem with a multitude of possible causes. There is evidence that pesticides may play a role, yet few pesticides have been tested on amphibians. For example, Roundup is a globally common herbicide that is conventionally thought to be nonlethal to amphibians. However, Roundup has been tested on few amphibian species, with existing tests conducted mostly under laboratory conditions and on larval amphibians. Recent laboratory studies have indicated that Roundup may be highly lethal to North American tadpoles, but we need to determine whether this effect occurs under more natural conditions and in post-metamorphic amphibians. I assembled communities of three species of North American tadpoles in outdoor pond mesocosms that contained different types of soil (which can absorb the pesticide) and applied Roundup as a direct overspray. After three weeks, Roundup killed 96–100% of larval amphibians (regardless of soil presence). I then exposed three species of juvenile (post-metamorphic) anurans to a direct overspray of Roundup in laboratory containers. After one day, Roundup killed 68–86% of juvenile amphibians. These results suggest that Roundup, a compound designed to kill plants, can cause extremely high rates of mortality to amphibians that could lead to population declines.

Artigo completo disponível em http://pbadupws.nrc.gov/docs/ML1434/ML14345A564.pdf

Oliveira, A.; Telles, L.; Hess, R.; Mahecha, G.; Oliveira, C. 2007. Effects of the herbicide Roundup on the epididymal region of drakes *Anas platyrhynchos. Reproductive Toxicology*, 23, 182–191.

Exposure to the Roundup has been shown to affect StAR protein and aromatase expression and activity, pointing out that this herbicide may cause adverse effects in animal reproduction by affecting androgen and estrogen synthesis. We tested this hypothesis by investigating the in vivo effects of the Roundup on the testis and epididymal region of drake Anas platyrhynchos. The exposure to the herbicide resulted in alterations in the structure of the testis and epididymal region as well as in the serum levels of testosterone and estradiol, with changes in the expression of androgen receptors restricted to the testis. The harmful effects were more conspicuous in the proximal efferent ductules and epididymal ducts, suggesting higher sensitivity of these segments among the male genital organs. The effects were mostly dose dependent, indicating that this herbicide may cause disorder in the morphophysiology of the male genital system of animals.

http://www.ncbi.nlm.nih.gov/pubmed/17166697

Soso, A.; Barcellos, L.; Ranzani-Paiva, M.; Kreutz, L.; Quevedo, R.; Anziliero, D.; Lima, M.; Silva, L.; Ritter, F.; Bedin, A.; Finco, J. 2007. Chronic exposure to sub-lethal concentration of a glyphosate based herbicide alters hormone profiles and affects reproduction of female Jundiá (*Rhamdia quelen*). *Environmental Toxicology and Pharmacology*, 23, 308-313.

This work was carried out to verify the effect of a glyphosate-based herbicide on Jundiá hormones (cortisol, 17β-estradiol and testosterone), oocyte and swim-up fry production. Earthen ponds containing Jundiá females were contaminated with glyphosate (3.6mg/L); blood samples were collected from eight females from each treatment immediately before, or at 1, 10, 20, 30 and 40 days following contamination. A typical post-stress rise in cortisol levels was observed at the 20th and 40th days following exposure to glyphosate. At the 40th day, 17β-estradiol was decreased in the exposed females. A similar number of oocytes were stripped out from females from both groups; however, a lower number of viable swim-up fry were obtained from the herbicide exposed females, which also had a higher liver-somatic index (LSI). The results indicate that the presence of glyphosate in water was deleterious to Rhamdia quelen reproduction, altering steroid profiles and egg viability.

http://www.ncbi.nlm.nih.gov/pubmed/21783773

Bringolf, R.; Cope, W.; Mosher, S.; Barnhart, M.; Shea, D. 2007. Acute and chronic toxicity of glyphosate compounds to *glochidia* and juveniles of *Lampsilis siliquoidea* (*Unionidae*). *Environ Toxicol Chem*, 26(10):2094-100.

Native freshwater mussels (family Unionidae) are among the most imperiled faunal groups in the world. Factors contributing to the decline of mussel populations likely include pesticides and other aquatic contaminants; however, there is a paucity of data regarding the toxicity of even the most globally distributed pesticides, including glyphosate, to mussels. Therefore, the toxicity of several forms of glyphosate, its formulations, and a surfactant (MON 0818) used in several glyphosate formulations was determined for early life stages of Lampsilis siliquoidea, a native freshwater mussel. Acute and chronic toxicity tests were performed with a newly established American Society of Testing and Materials (ASTM) standard guide for conducting toxicity tests with freshwater mussels. Roundup, its active ingredient, the technical-grade isopropylamine (IPA) salt of glyphosate, IPA alone, and MON 0818 (the surfactant in Roundup formulations) were each acutely toxic to L. siliquoidea glochidia. MON 0818 was most toxic of the compounds tested and the 48-h median effective concentration (0.5 mg/L) for L. siliquoidea glochidia is the lowest reported for any aquatic organism tested to date. Juvenile L. siliquoidea were also acutely sensitive to MON 0818, Roundup, glyphosate IPA salt, and IPA alone. Technical-grade glyphosate and Aqua Star were not acutely toxic to glochidia or juveniles. Ranking of relative chronic toxicity of the glyphosate-related compounds to juvenile mussels was similar to the ranking of relative acute toxicity to juveniles. Growth data from chronic tests was largely inconclusive. In summary, these results indicate that L. siliquoidea, a representative of the nearly 300 freshwater mussel taxa in North America, is among the most sensitive aquatic organisms tested to date with glyphosate-based chemicals and the surfactant MON 0818.

http://www.ncbi.nlm.nih.gov/pubmed/17867870

Glusczak, L.; Miron, D.; Moraes, B.; Simões, R.; Schetinger, M.; Morsch, V.; Loro, V. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, Volume 146, Issue 4, Pages 519–524.

Silver catfish (*Rhamdia quelen*; Teleostei) were exposed to commercial formulation Roundup®, a glyphosate herbicide: 0 (control), 0.2 or 0.4 mg/L for 96 h. Fish exposed to glyphosate showed an increase in hepatic glycogen, but a reduction in muscle glycogen at both concentrations tested. Glucose decreased in liver and increased in muscle of fish at both herbicide concentrations.

Glyphosate exposure increased lactate levels in liver and white muscle at both concentrations. Protein levels increased in liver and decreased in white muscle while levels of ammonia in both tissues increased in fish at both glyphosate concentrations. Specific AChE activity was reduced in brain after treatments, no changes were observed in muscle tissue. Catalase activity in liver did not change during of exposure. Fish exposed to glyphosate demonstrated increased TBARS production in muscle tissue at both concentrations tested. For both glyphosate concentrations tested brain showed a reduction of TBARS after 96 h of exposure. The present results showed that in 96 h, glyphosate changed AChE activity, metabolic parameters and TBARS production. The parameters measured can be used as herbicide toxicity indicators considering environmentally relevant concentration.

http://www.sciencedirect.com/science/article/pii/S1532045607001470

Langiano, V.; Martinez, C. 2008. Toxicity and effects of a glyphosate-based herbicide on the neotropical fish Prochilodus lineatus. *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology*, 147, 222-231.

The toxicity of Roundup, a glyphosate-based herbicide widely used in agriculture, was determined for the Neotropical fish *Prochilodus lineatus*. The 96 h-LC_{50} of Roundup was 13.69 mg L^{-1}, indicating that this fish is more sensitive to Roundup than rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). These differences should be considered when establishing criteria for water quality and animal well-being in the Neotropical region. Short-term (6, 24 and 96 h) toxicity tests were then performed to evaluate the effects of sub-lethal concentrations of the herbicide (7.5 and 10 mg L^{-1}) to *P. lineatus*. Roundup did not interfere with the maintenance of the ionic balance and there was no significant alteration in plasma cortisol levels in Roundup-exposed fish. However an increase in plasma glucose was noted in fish exposed to 10 mg L^{-1} of the herbicide, indicating a typical stress response. Catalase liver activity also showed an increase in fish exposed to 10 mg L^{-1} of the herbicide, suggesting the activation of antioxidant defenses after Roundup exposure. In addition, Roundup induced several liver histological alterations that might impair normal organ functioning. Therefore, short-term exposure to Roundup at subletal concentrations induced biochemical, physiological and histological alterations in *P. lineatus*.

http://www.sciencedirect.com/science/article/pii/S1532045607002086

Cavalcante, D.; Martinez, C.; Sofia, S. 2008. Genotoxic effects of Roundup® on the fish *Prochilodus lineatus. Mutation Research*, 655 (2008) 41–46.

Glyphosate-based herbicides, such as Roundup, represent the most extensively used herbicides worldwide, including Brazil. Despite its extensive use, the genotoxic effects of this herbicide are not completely understood and studies with Roundup show conflicting results with regard to the effects of this product on the genetic material. Thus, the aim of this study was to evaluate the genotoxic effects of acute exposures (6, 24 and 96 h) to 10 mg L(-1) of Roundup on the neotropical fish Prochilodus lineatus. Accordingly, fish erythrocytes were used in the comet assay, micronucleus test and for the analysis of the occurrence of nuclear abnormalities and the comet assay was adjusted for branchial cells. The results showed that Roundup produces genotoxic damage in erythrocytes and gill cells of P. lineatus. The comet scores obtained for P. lineatus erythrocytes after 6 and 96 h of exposure to Roundup were significantly higher than respective negative controls. For branchial cells comet scores were significantly higher than negative controls after 6 and 24 h exposures. The frequencies of micronucleus and other erythrocyte nuclear abnormalities (ENAs) were not significantly different between Roundup exposed fish and their respective negative controls, for all exposure periods. In conclusion, the results of this work showed that Roundup produced genotoxic effects on the fish species P. lineatus. The comet assay with gill cells showed to be an important complementary tool for detecting genotoxicity, given that it revealed DNA damage in periods of exposure that erythrocytes did not. ENAs frequency was not a good indicator of genotoxicity, but further studies are needed to better understand the origin of these abnormalities.

http://www.ncbi.nlm.nih.gov/pubmed/18638566

Relyea, R.; Jones, D. 2009. The toxicity of Roundup Original Max to 13 species of larval amphibians. *Environ Toxicol Chem*, 28(9):2004-8.

With the increased use of glyphosate-based herbicides (marketed under several names, including Roundup and Vision), there has been a concomitant increased concern about the unintended impacts that particular formulations containing the popular surfactant polyethoxylated tallowamine (POEA) might have on amphibians. Published studies have examined a relatively small number of anuran species (primarily from Australia and eastern North America) and, surprisingly, no species of salamanders. Using a popular formulation of glyphosate (Roundup Original Max), the goal of the present study was to conduct tests of lethal concentrations estimated to kill 50% of a population after 96 h (LC50(96-h)) on a wider diversity of species from both eastern and western North America. Tests were conducted on nine species of stage 25, larval anurans from three families (Ranidae: Rana pipiens, R. clamitans, R. sylvatica, R. catesbeiana, R. cascadae; Bufonidae: Bufo americanus, B. boreas; and Hylidae: Hyla versicolor, Pseudacris crucifer) and four species of larval salamanders from two families (Ambystomatidae: Ambystoma gracile, A. maculatum, A. laterale; and Salamandridae: Notophthalmus viridescens). For the nine species of larval anurans, LC50(96-h) values ranged from 0.8- to 2.0-mg acid equivalents per liter with relatively little pattern in differential sensitivity among the species or families. The four species of larval salamanders were less sensitive than the anurans, with LC50(96-h) values ranging from 2.7- to 3.2-mg acid equivalents per liter and no substantial differences among the species of salamanders. This work substantially increases the available data on amphibian sensitivity to glyphosate formulations that include either POEA surfactants or the equally moderately to highly toxic surfactants of Roundup Original Max and should be useful for improving future risk assessments.

http://www.ncbi.nlm.nih.gov/pubmed/19405783

Senapati, T.; Mukerjee, A.; Ghosh, A. 2009. Observations on the effect of glyphosate based herbicide on ultra structure (SEM) and enzymatic activity in different regions of alimentary canal and gill of *Channa punctatus* (Bloch). *Journal of Crop and Weed*, 5(1): 236–245.

Glyphosate is the isopropyl amine salt of N-(Phosphonomethyl)-glycine, a broad-spectrum nonselective herbicide, which has been extensively used to control annual and perennial weeds in agricultural, forest and aquatic systems. The ultrastructural changes in different regions of alimentary canal and gill were observed by Scanning Electron Microscopic study on a non-target aquatic teleostea fish, Channa punctatus. Fishes were exposed to herbicide at a dose of 4 mgl^{-1} generally used by farmers to control weeds in water bodies for a period of 45 days in laboratory condition with a control. Severe damage, shrinkage and degeneration of pentagonal cellular contour of stratified epithelial cells (SEC) were observed in gill. Shrinkage of SEC resulting in degeneration of microridges was observed in buccopharynx. Slight necrosed and distorted SEC was observed in oesophagus. Severe mucus secretion was observed in stomach. Erosion on the apical surface of mucosal folds and columnar epithelial cells (CEC) and necrosis of CEC was also noticed in stomach. Obliteration of CEC along its entire length from basement membrane was observed in the intestinal portion. After 45 days treatment by glyphosate protease activity was slightly reduced in stomach and intestine in comparison to control fish. Amylase activity reduced in oesophagus and intestine in treated condition. Lipase activity was also reduced slightly in stomach and intestine of glyphosate treated fish.

http://cropandweed.com/vol5issue1/46.1.html

Albinati, A.; Moreira, E.; Albinati, R.; Carvalho, J.; Lira, A.; Santos, G.; Vida, L. 2009. Biomarcadores histológicos – toxicidade crônica pelo Roundup em piauçu (*Leporinus macrocephalus*). *Arq Bras Med Vet Zootec*, 61(3): 621–627.

Avaliou-se o uso de brânquias, fígado e rins como biomarcadores histológicos em ensaio de toxicidade crônica com o herbicida Roundup® em piauçu (L*eporinus. macrocephalus*). Para tanto, os animais foram expostos a 1,58mg/L, dose equivalente a 1/10 da CL50 para a espécie por 14 e 28 dias, sendo utilizados cinco animais por tratamento correspondentes aos dias 0, 14 e 28. Hemorragia e necrose hepática e congestão renal foram as alterações que apresentaram diferenças entre os animais expostos e os não expostos. Dentre os órgãos usados como biomarcadores histopatológicos, o fígado foi o que apresentou os melhores resultados, seguido pelo rim.

Artigo completo disponível em https://repositorio.ufba.br/ri/bitstream/ri/6321/1/Albinati,%20A.C.L..pdf

Modesto, K.; Martinez, C. 2010. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere*, Volume 78, Issue 3, Pages 294–299.

This work aimed to evaluate Roundup® effects on biochemical biomarkers of the neotropical fish *Prochilodus lineatus*. Fish were acutely exposed (6, 24 and 96 h) to 10 mg L^{-1} of Roundup® (RD) or only water (control) and samples of liver, for antioxidants analysis, and brain and muscle, for acetylcholinesterase (AChE) determination, were collected. Fish exposed to RD for 24 h showed reduction on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, and increased glutathione (GSH) content. After 24 and 96 h, fish of RD group showed increased glutathione-S-transferase (GST) activity and lipid peroxidation. AChE activity was inhibited in brain after 96 h and in muscle after 24 and 96 h of exposure. Thus, acute exposure to RD stimulated the biotransformation pathway, with increased GST, but interfered on the antioxidant defenses, with reduction of SOD and GPx activity, leading to the occurrence of lipid peroxidation. Inhibition of AChE showed that RD acts as a contaminant with anti-AChE action.

http://www.sciencedirect.com/science/article/pii/S0045653509012739

Jayawardena, U.; Rajakaruna, R.; Navaratne, A.; Amerrasinghe, P. 2010. Toxicity of agrochemicals to common hourglass tree frog (*Polypedates cruciger*) in acute and chronic exposure. *International Journal of Agriculture and Biology*, 12, 641-648.

Direct effect of four common agricultural pesticides viz., chlorpyrifos, dimethoate, glyphosate and propanil, on the survival, growth and development of malformations in common hourglass tree frog, *Polypedates cruciger* (Anura: Ranidae) was studied under laboratory conditions in acute and chronic exposure. Acute exposure to high concentrations was carried out to determine the LC50. The 48 h LC50 of the pesticides were within the Pesticide Area Network specified limits, except for propanil. The percentage survival of the tadpoles under chronic exposure to ecologically relevant doses was lower (glyphosate 75%, dimethoate 77.5%, chlorpyrifos 80% & propanil 85%) than the control group (95.5%) and was significantly affected by the concentrations. Exposed tadpoles took more time to metamorphose and were significantly smaller in size than the control tadpoles. They also developed malformations at high frequencies (glyphosate = 69%, dimethoate = 64%, chlorpyrifos = 60%, propanil = 45%). Malformations were mainly kyphosis (hunched back), scoliosis (curvature), skin ulcers and edema. However, severe limb malformations were not observed in the study. Chlorpyrifos had a profound effect even at very low concentrations (0.05 ppm). This study provides the first empirical evidence of a comparative study on the effect of pesticides on an endemic amphibian species in Sri Lanka and underscores the importance of investigation the level of agricultural pesticides in freshwater ecosystems and their effect on non-target organisms.

Artigo completo disponível em http://www.fspublishers.org/published_papers/28756_..pdf

Kelly, D.; Poulin, R.; Tompkins, D.; Townsend, C. 2010. Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. *Journal of Applied Ecology*, 47, 498-504.

1. Anthropogenic pollution and disease can cause both lethal and sub-lethal effects in aquatic species but our understanding of how these stressors interact is often not known. Contaminants can reduce host resistance to disease, but whether hosts are impacted at environmentally relevant concentrations is poorly understood.
2. We investigated the independent and combined effects of exposure to the common herbicide glyphosate and the trematode parasite Telogaster opisthorchis on survival and the development of spinal malformations in juvenile Galaxias anomalus, a New Zealand freshwater fish. We then investigated how exposure to a glyphosate concentration gradient (0Æ36, 3Æ6, 36 mg active ingrediente (a.i.) L)1) affected the production and release of the infective cercarial stage of the parasite by its snail intermediate host Potamopyrgus antipodarum.
3. Survival of juvenile fish was unaffected by exposure to glyphosate alone (at an environmentally relevant concentration; 0Æ36 mg a.i. L)1) or by T. opisthorchis infection alone. However, simultaneous exposure to infection and glyphosate significantly reduced fish survival.
4. Juvenile fish developed spinal malformations when exposed either to infections alone or to infections and glyphosate, with a trend towards greater severity of spinal malformation after exposure to both stressors.
5. All snails exposed to the highest glyphosate concentration (36 mg a.i. L)1) died within 24 h. Snails exposed to a moderate concentration (3Æ6 mg a.i. L)1) produced significantly more T. opisthorchis cercariae than snails in the control group or the low concentration group (0Æ36 mg a.i. L)1; the same concentration as in the fish experiment).
6. Synthesis and applications. This is the first study to show that parasites and glyphosate can act synergistically on aquatic vertebrates at environmentally relevant concentrations, and that glyphosate might increase the risk of disease in fish. Our results have important implications when identifying risks to aquatic communities and suggest that threshold levels of glyphosate currently set by regulatory authorities do not adequately protect freshwater systems.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2010.01791.x/pdf

Relyea, R. 2012. New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. *Ecological Applications*, 22(2), 2012, pp. 634–647.

The use of pesticides is important for growing crops and protecting human health by reducing the prevalence of targeted pest species. However, less attention is given to the potential unintended effects on nontarget species, including taxonomic groups that are of current conservation concern. One issue raised in recent years is the potential for pesticides to become more lethal in the presence of predatory cues, a phenomenon observed thus far only in the laboratory. A second issue is whether pesticides can induce unintended trait changes in nontarget species, particularly trait changes that might mimic adaptive responses to natural environmental stressors. Using outdoor mesocosms, I created simple wetland communities containing leaf litter, algae, zooplankton, and three species of tadpoles (wood frogs [*Rana sylvatica* or *Lithobates sylvaticus*], leopard frogs [*R. pipiens* or *L. pipiens*], and American toads [*Bufo americanus* or *Anaxyrus americanus*]). I exposed the communities to a factorial combination of environmentally relevant herbicide concentrations (0, 1, 2, or 3 mg acid equivalents [a.e.]/L of Roundup Original MAX) crossed with three predator-cue treatments (no predators, adult newts [*Notophthalmus viridescens*], or larval dragonflies [*Anax junius*]). Without predator cues, mortality rates from Roundup were consistent with past studies. Combined with cues from the most risky predator (i.e., dragonflies), Roundup became less lethal (in direct contrast to past laboratory studies). This reduction in mortality was likely caused by the herbicide stratifying in the water column and predator cues scaring the tadpoles down to the benthos where herbicide concentrations were lower. Even more striking was the discovery that Roundup induced morphological changes in the tadpoles. In wood frog and leopard frog tadpoles, Roundup induced relatively deeper tails in the same direction and of the same magnitude as the adaptive changes induced by dragonfly cues. To my knowledge, this is the first study to show that a pesticide can induce morphological changes in a vertebrate. Moreover, the data suggest that the herbicide might be activating the tadpoles' developmental pathways used for antipredator responses. Collectively, these discoveries suggest that the world's most widely applied herbicide may have much further-reaching effects on nontarget species than previous considered.

http://www.esajournals.org/doi/abs/10.1890/11-0189.1

Mottier, A.; Kientz-Bouchart, V.; Serpentini, A.; Lebel, J; Jha, A.; Costil, K. 2013. Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*. *Aquat Toxicol*,15;128-129:67-78. doi: 10.1016.

Pesticides may be involved in oyster summer mortality events, not necessarily as a single causative agent but as an additional stressor. In this context, the present study aimed to assess the toxicity of glyphosate, its by-product, aminomethylphosphonic acid (AMPA) and two commercial formulations, Roundup Express(®) (R(EX)) and Roundup Allées et Terrasses(®) (R(AT)), containing glyphosate as the active ingredient, on the early life stages of the Pacific oyster, Crassostrea gigas. The embryotoxicity of these chemicals were quantified by considering both the rates of abnormalities and the arrested development or types of abnormalities in D-shaped larvae after 48 h exposure. The success of metamorphosis was examined in pediveliger larvae exposed for 24 h. Experiments involving both endpoints included range finding experiments for herbicide concentrations ranging from 0.1 to 100,000 μg L(-1). This range was then narrowed down in order to determine precise EC(50) values. Actual concentrations of the herbicide were determined at the beginning and after 48 h (embryotoxicity) and 24 h (metamorphosis) to evaluate the potential temporal variation in the concentrations. During embryo-larval development, no mortalities were recorded at any of the concentrations of glyphosate and AMPA, whereas no embryos or D-shaped larvae could be observed after exposure to 10,000 μg L(-1) of R(EX) or R(AT). Compared with the controls, no effects on embryo-larval development were recorded between 0.1 and 1000 μg L(-1), regardless of the chemical tested. Above a threshold, which varied according to the chemical used, the gradient of herbicide concentrations correlated with a gradient of severity of abnormality ranging from normal larvae to arrested development (an "old embryo" stage). The EC(50) values were 28,315 and 40,617 μg L(-1) for glyphosate and its metabolite, respectively, but much lowered values of 1133 and 1675 μg L(-1) for R(EX) and R(AT), respectively. Metamorphosis tests also revealed a significant difference between molecules, as the EC(50) values exceeded 100,000 μg L(-1) for glyphosate and AMPA but were as low as 6366 and 6060 μg L(-1) for the commercial formulations, which appeared relatively more toxic. Overall, the embryo-larval development of C. gigas was more sensitive to glyphosate-based herbicides compared to various endpoints studied in regulatory model organisms, and embryos and D-shaped larvae were more sensitive compared to pediveliger larvae.

http://www.ncbi.nlm.nih.gov/pubmed/23277103

Cuhra, M.; Traavik, T.; Bøhn, T. 2013. Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. *Ecotoxicology*, 22 (2), 251-262. (DOI: 10.1007/s10646-012-1021-1).

Low levels of glyphosate based herbicide induced significant negative effects on the aquatic invertebrate Daphnia magna. Glyphosate herbicides such as brands of Roundup, are known to be toxic to daphnids. However, published findings on acute toxicity show significant discrepancies and variation across several orders of magnitude. To test the acute effects of both glyphosate and a commercial formulation of Roundup (hereafter Roundup), we conducted a series of exposure experiments with different clones and age-classes of D. magna. The results demonstrated EC(50) (48) values in the low ppm-range for Roundup as well as for the active ingredient (a.i.) isopropylamine salt of glyphosate (glyphosate IPA) alone. Roundup showed slightly lower acute toxicity than glyphosate IPA alone, i.e. EC(50) values of 3.7-10.6 mg a.i./l, as compared to 1.4-7.2 mg a.i./l for glyphosate IPA. However, in chronic toxicity tests spanning the whole life-cycle, Roundup was more toxic. D. magna was exposed to sublethal nominal concentrations of 0.05, 0.15, 0.45, 1.35 and 4.05 mg a.i./l for 55 days. Significant reduction of juvenile size was observed even in the lowest test concentrations of 0.05 mg a.i./l, for both glyphosate and Roundup. At 0.45 mg a.i./l, growth, fecundity and abortion rate was affected, but only in animals exposed to Roundup. At 1.35 and 4.05 mg a.i./l of both glyphosate and Roundup, significant negative effects were seen on most tested parameters, including mortality. D. magna was adversely affected by a near 100 % abortion rate of eggs and embryonic stages at 1.35 mg a.i./l of Roundup. The results indicate that aquatic invertebrate ecology can be adversely affected by relevant ambient concentrations of this major herbicide. We conclude that glyphosate and Roundup toxicity to aquatic invertebrates have been underestimated and that current European Commission and US EPA toxicity classification of these chemicals need to be revised.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3572389/

Farabaugh, N.; Nowakowski, A. 2014. Behavioral responses of the strawberry poison frog (*Oophaga pumilio*) to herbicide olfactory cues: possible implications for habitat selection and movement in altered landscapes. *Canadian Journal of Zoology,* 92, 979-984.

Recent research has focused on the importance of behavior in mediating the effects of landscape change on amphibian populations and communities. Factors such as chemical contaminants may affect habitat selection and movement of amphibians in human-altered habitats and contribute to landscape-level patterns of distribution and abundance. The objective of this study was to determine if the Strawberry Poison Frog (*Oophaga pumilio* (Schmidt, 1857)) can use olfactory cues to detect and avoid the glyphosate-based herbicide Roundup™. Fifty frogs were captured in the field in Costa Rica and tested in experimental arenas where they were given a choice between a control and an herbicide treatment. Analysis of time spent in treatment areas revealed a significant interaction between sex and treatment. Analyses of choice at the start and end of the trials indicated that sex and cardinal direction were important factors influencing orientation behavior. These results suggest that males and females differed in their behavioral responses, and that male *O. pumilio* may use olfactory cues to detect and avoid areas treated with glyphosate-based herbicide. However, the sampled population was male-biased, which resulted in a lower sample size and lower power to detect an effect for females. Further work is needed to better understand amphibian behavioral responses to herbicides, as well as the role of sex and individual variation in modifying these responses.

http://www.nrcresearchpress.com/doi/abs/10.1139/cjz-2014-0111

Lanctôt, C.; Navarro-Martín, L.; Robertson, C.; Park, B.; Jackman, P.; Pauli, B.; Trudeau, V. 2014. Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frog (*Lithobates sylvaticus*) tadpoles. II: Agriculturally relevant exposures to Roundup WeatherMax® and Vision® under laboratory conditions. *Aquatic Toxicology,* 154, 291-303.

Glyphosate-based herbicides are currently the most commonly used herbicides in the world. They have been shown to affect survival, growth, development and sexual differentiation of tadpoles under chronic laboratory exposures but this has not been investigated under more environmentally realistic conditions. The purpose of this study is (1) to determine if an agriculturally relevant exposure to Roundup WeatherMax®, a relatively new and understudied formulation, influences the development of wood frog tadpoles (*Lithobates sylvaticus*) through effects on the mRNA levels of genes involved in the control of metamorphosis; (2) to compare results to the well-studied Vision® formulation (containing the isopropylamine salt of glyphosate [IPA] and polyethoxylated tallowamine [POEA] surfactant) and to determine which ingredient(s) in the formulations are responsible for potential effects on development; and (3) to compare results to recent field studies that used a similar experimental design. In the present laboratory study, wood frog tadpoles were exposed to an agriculturally relevant application (i.e., two pulses) of Roundup WeatherMax® and Vision® herbicides as well as the active ingredient (IPA) and the POEA surfactant of Vision®. Survival, development, growth, sex ratios and mRNA levels of genes involved in tadpole metamorphosis were measured. Results show that Roundup WeatherMax® (2.89 mg acid equivalent (a.e.)/L) caused 100% mortality after the first pulse. Tadpoles treated with a lower concentration of Roundup WeatherMax® (0.21 mg a.e./L) as well as Vision® (2.89 mg a.e./L), IPA and POEA had an increased condition factor (based on length and weight measures in the tadpoles) relative to controls at Gosner stage (Gs) 36/38. At Gs42, tadpoles treated with IPA and POEA had a decreased condition factor. Also at Gs42, the effect on condition factor was dependent on the sex of tadpoles and significant treatment effects were only detected in males. In most cases, treatment reduced the normal mRNA increase of key genes controlling development in tadpoles between Gs37 and Gs42, such as genes encoding thyroid hormone receptor beta in brain, glucocorticoid receptor in tail and deiodinase enzyme in brain and tail. We conclude that glyphosate-based herbicides have the potential to alter mRNA profiles during metamorphosis. However, studies in natural systems have yet to replicate these negative effects, which highlight the need for more ecologically relevant studies for risk assessment.

http://www.sciencedirect.com/science/article/pii/S0166445X1400191X

Navarro-Martín, L.; Lanctôt, C.; Jackman, P.; Park, B.; Doe, K.; Pauli, B.; Trudeau, V. 2014. Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (*Lithobates sylvaticus*) tadpoles. I: Chronic laboratory exposures to VisionMax®. *Aquatic Toxicology,* 154, 278-290.

The purpose of this study was to determine if chronic exposure to the glyphosate-based herbicide VisionMax® affects the survival, development, growth, sex ratios and expression of specific genes involved in metamorphosis of wood frog tadpoles (*Lithobates sylvaticus*). We hypothesized that exposure to this herbicide will affect developmental rates by disrupting hormone pathways, sex ratios and/or gonadal morphology. Tadpoles were chronically exposed in the laboratory from Gosner developmental stage 25 to 42 to four different concentrations of VisionMax® (ranging from 0.021 to 2.9 mg acid equivalents/L). Chronic exposures to VisionMax® had direct effects on the metamorphosis of *L. sylvaticus* tadpoles by decreasing development rates, however, there was a decrease in survival only in the group exposed to the highest dose of VisionMax® (2.9 mg a.e./L; from approximately 96% in the control group to 77% in the treatment group). There was a decrease in the number of tadpoles reaching metamorphic climax, from 78% in the control group to 42% in the VisionMax® (2.9 mg a.e./L) group, and a 7-day delay to reach metamorphic climax in the same treatment group. No effects of exposure on sex ratios or gonadal morphology were detected in tadpoles exposed to any of the concentrations of VisionMax® tested. Gene expression analyses in brain and tail tissues demonstrated that exposure to VisionMax® alters the expression of key genes involved in development. Results showed significant interaction (two-way ANOVA, $P < 0.05$) between developmental Gosner stage and treatment in brain *corticotropin-releasing factor*, deiodinase type II (*dio2*) and *glucocorticotiroid receptor* (*grII*) and tail *dio2* and *grII*. This demonstrates that mRNA levels may be differently affected by treatment depending on the developmental stage at which they are assessed. At the same time there was a clear dose–response effect for VisionMax® to increase thyroid hormone receptor β in tadpole brain ($F_{(2,69)} = 3.475$, $P = 0.037$) and tail ($F_{(2,69)} = 27.569$, $P < 0.001$), regardless of developmental stage. Interestingly, delays in development (or survival) were only observed in the group exposed to 2.9 mg a.e./L of VisionMax®, suggesting that tadpoles need to be exposed to a “threshold” concentration of glyphosate-based herbicide to exhibit phenotypic observable effects. We suggest that the upregulation of genes that trigger metamorphosis following VisionMax® herbicide exposure might result from a compensatory response for the delays in development observed. Further studies are needed to determine if disruption of expression of these key genes leads to long-term effects when metamorphs reach adult stages.

http://www.sciencedirect.com/science/article/pii/S0166445X14001763

Moreno, N.; Sofia, S.; Martinez C. 2014. Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environ Toxicol Pharmacol*, 37(1):448-54. doi: 10.1016/j.etap.2013.12.012.

Roundup Transorb (RT) is a glyphosate-based herbicide and despite its wide use around the world there are few studies comparing the effects of the active ingredient with the formulated product. In this context the purpose of this study was to compare the genotoxicity of the active ingredient glyphosate with the formulated product RT in order to clarify whether the active ingredient and the surfactant of the RT formula may exert toxic effects on the DNA molecule in juveniles of fish Prochilodus lineatus. Erythrocytes and gill cells of fish exposed to glyphosate and to RT showed DNA damage scores significantly higher than control animals. These results revealed that both glyphosate itself and RT were genotoxic to gill cells and erythrocytes of P. lineatus, suggesting that their use should be carefully monitored considering their potential impact on tropical aquatic biota.

http://www.ncbi.nlm.nih.gov/pubmed/24448465

Zaller, J.; Heigl, F.; Ruess, L.; Grabmaier, A. 2014. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Scientific Reports*, 4, Article number: 5634. doi:10.1038/srep05634.

Herbicides containing glyphosate are widely used in agriculture and private gardens, however, surprisingly little is known on potential side effects on non-target soil organisms. In a greenhouse experiment with white clover we investigated, to what extent a globally-used glyphosate herbicide affects interactions between essential soil organisms such as earthworms and arbuscular mycorrhizal fungi (AMF). We found that herbicides significantly decreased root mycorrhization, soil AMF spore biomass, vesicles and propagules. Herbicide application and earthworms increased soil hyphal biomass and tended to reduce soil water infiltration after a simulated heavy rainfall. Herbicide application in interaction with AMF led to slightly heavier but less active earthworms. Leaching of glyphosate after a simulated rainfall was substantial and altered by earthworms and AMF. These sizeable changes provide impetus for more general attention to side-effects of glyphosate-based herbicides on key soil organisms and their associated ecosystem services.

Artigo completo disponível em http://www.nature.com/srep/2014/140709/srep05634/full/srep05634.html

Como discutido em documentos organizados na Parte 3 dessa publicação (referente aos impactos das plantas transgênicas para a saúde humana e animal), o perfil ecotoxicológico dos herbicidas à base de glifosato não se faz adequadamente representado pelo perfil ecotoxicológico do seu princípio ativo glifosato. Os produtos comerciais, as misturas de componentes e mesmo os metabólitos de degradação mostram-se severamente mais danosos que o princípio ativo, com riscos toxicológicos e biológicos bem mais expressivos. Os produtos realmente aplicados nos agrossistemas sempre envolvem xaropes em que o princípio ativo depende de adjuvantes que, além de aportarem sua própria toxicidade, contribuem para potencializar (via efeitos sinérgicos) os impactos relacionados ao princípio ativo em si.

Em outras palavras, como o glifosato não atua de forma isolada são de escassa validade as análises que desconsideram esse fato. Ainda assim, mesmo o glifosato em particular se revela extremamente danoso, sendo alarmante o fato de que agências reguladoras tratem de dissociar seus impactos de análises do risco que examinam plantas GM TH a ele associadas.

Este capítulo reúne artigos que demonstram ser mais graves os impactos ambientais decorrentes do uso do produto comercial Roundup do que aqueles associados a seu princípio ativo, o glifosato.

Cabe mencionar que os testes toxicológicos realizados em laboratório para avaliação do agrotóxico, quando descartam o produto comercial e se fixam no princípio ativo, mistificam os resultados, minimizando efeitos que seguramente ocorrerão no mundo real.

Tsui, M.; Chu, L. 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere*, 52(7):1189-97.

Glyphosate-based herbicides (e.g. Roundup) are extensively used in the aquatic environment, but there is a paucity of data on the toxicity of the formulated products and the influences by environmental factors. In this study, the acute toxicity of technical-grade glyphosate acid, isopropylamine (IPA) salt of glyphosate, Roundup and its surfactant polyoxyethylene amine (POEA) to Microtox bacterium (Vibrio fischeri), microalgae (Selenastrum capricornutum and Skeletonema costatum), protozoa (Tetrahymena pyriformis and Euplotes vannus) and crustaceans (Ceriodaphnia dubia and Acartia tonsa) was examined and the relative toxicity contributions of POEA to Roundup were calculated. The effects of four environmental factors (temperature, pH, suspended sediment and algal food concentrations) on the acute toxicity of Roundup to C. dubia were also examined. Generally, the toxicity order of the chemicals was: POEA>Roundup>glyphosate acid>IPA salt of glyphosate, while the toxicity of glyphosate acid was mainly due to its high acidity. Microtox bacterium and protozoa had similar sensitivities towards Roundup toxicity (i.e. IC50 from 23.5 to 29.5 mg AE/l). In contrast, microalgae and crustaceans were 4-5 folds more sensitive to Roundup toxicity than bacteria and protozoa. Except photosynthetic microalgae, POEA accounted for more than 86% of Roundup toxicity and the toxicity contribution of POEA was shown to be species-dependent. Increase in pH (6-9) and increase of suspended sediment concentration (0-200 mg/l) significantly increased the toxicity of Roundup to C. dubia, but there were no significant effects due to temperature change and food addition.

http://www.ncbi.nlm.nih.gov/pubmed/12821000

Howe, C.; Berrill, M.; Pauli, B.; Helbing, C.; Werry, K.; Veldhoen, N. 2004. Toxicity of glyphosate-based pesticides to four North American frog species. *Environ Toxicol Chem*, 23(8):1928-38.

Glyphosate-based herbicides are among the most widely used pesticides in the world. We compared the acute toxicity of the glyphosate end-use formulation Roundup Original to four North American amphibian species (Rana clamitans, R. pipiens, R. sylvatica, and Bufo americanus) and the toxicity of glyphosate technical, the polyethoxylated tallowamine surfactant (POEA) commonly used in glyphosate-based herbicides, and five newer glyphosate formulations to R. clamitans. For R. clamitans, acute toxicity values in order of decreasing toxicity were POEA > Roundup Original > Roundup Transorb > Glyfos AU; no significant acute toxicity was observed with glyphosate technical material or the glyphosate formulations Roundup Biactive, Touchdown, or Glyfos BIO. Comparisons between the four amphibian species showed that the toxicity of Roundup Original varied with species and developmental stage. Rana pipiens tadpoles chronically exposed to environmentally relevant concentrations of POEA or glyphosate formulations containing POEA showed decreased snout-vent length at metamorphosis and increased time to metamorphosis, tail damage, and gonadal abnormalities. These effects may be caused, in some part, by disruption of hormone signaling, because thyroid hormone receptor beta mRNA transcript levels were elevated by exposure to formulations containing glyphosate and POEA. Taken together, the data suggest that surfactant composition must be considered in the evaluation of toxicity of glyphosate-based herbicides.

http://www.ncbi.nlm.nih.gov/pubmed/15352482

Contardo-Jara, V.; Klingelmann, E.; Wiegand, C. 2009. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environmental Pollution*, 157 (1) 57–63.

The bioaccumulation potential of glyphosate and the formulation Roundup Ultra, as well as possible effects on biotransformation and antioxidant enzymes in Lumbriculus variegatus were compared by four days exposure to concentrations between 0.05 and 5 mg L(-1) pure glyphosate and its formulation. Bioaccumulation was determined using (14)C labeled glyphosate. The bioaccumulation factor (BCF) varied between 1.4 and 5.9 for the different concentrations, and was higher than estimated from logP(ow). Glyphosate and its surfactant POEA caused elevation of biotransformation enzyme soluble glutathione S-transferase at non-toxic concentrations. Membrane bound glutathione S-transferase activity was significantly elevated in Roundup Ultra exposed worms, compared to treatment with equal glyphosate concentrations, but did not significantly differ from the control. Antioxidant enzyme superoxide dismutase was significantly increased by glyphosate but in particular by Roundup Ultra exposure indicating oxidative stress. The results show that the formulation Roundup Ultra is of more ecotoxicological relevance than the glyphosate itself.

http://www.ncbi.nlm.nih.gov/pubmed/18790555

Lipok, J.; Studnik, H.; Gruyaert, S. 2010. The toxicity of Roundup® 360 SL formulation and its main constituents: glyphosate and isopropylamine towards non-target water photoautotrophs. *Ecotoxicol Environ Saf*, 73(7):1681-8. doi: 10.1016/j.ecoenv.2010.08.017.

The toxicity of commercial formulation of Roundup® 360 SL, widely used, nonselective herbicide and its main constituents, glyphosate (PMG), equimolar (1:1) isopropylamine salt of glyphosate (GIPA) and isopropylamine (IPA) was examined towards eight aquatic microphotoautotrophs; seven cyanobacterial strains representing either saline or freshwater communities, and common eukaryotic algae Chlorella vulgaris Beijerinck. Autotrophs were cultured 21 days in their appropriate standard media supplemented with various amounts of Roundup®, glyphosate, GIPA and IPA.

The determination of the growth of examined photoautotrophs was performed by time-course measurements of total chlorophyll content in experimental cultures. The growth rates related to corresponding concentrations of chemicals, the EC(50) values and generation doubling time were determined in order to present the toxicity Roundup® 360 SL formulation and its main constituents. Market available formulation of Roundup® was found to possess toxicity significantly higher than this, attributed to its main constituents; however both these compounds, isopropylamine and glyphosate, also inhibited the growth of examined strains in a dose-dependent manner. Notably, the interpretation of toxicity of the examined substances was found to be significantly dependent on the method of EC(50) calculation. The choice of molar or weight concentration of substances tested separately and in specific formulation was found to be essential in this matter. Due to these findings the EC(50) values were calculated based either on molar or on weight concentrations. Considering Roundup® 360 SL formulation, these values ranged from 10(-3) up to 10(-1) mM and they were one order of magnitude lower than those found for isopropylamine. Quite surprisingly the minimum EC(50) values found for glyphosate did not reach micromolar concentrations, whereas most of the EC(50) values revealed to IPA did not exceed this range. Notably, in all the cases except for Synechocystis aquatilis Sauvageau, isopropylamine alone was indicated as more toxic than glyphosate.

http://www.ncbi.nlm.nih.gov/pubmed/20813408

Moore, L.; Fuentes, L.; Rodgers, J.; Bowerman, W.; Yarrow, G.; Chao, W.; Bridges, W. 2012. Relative toxicity of the components of the original formulation of Roundup to five North American anurans. *Ecotoxicol Environ Saf*, 78:128-33. doi: 10.1016/j.ecoenv.2011.11.025.

The responses of five North American frog species that were exposed in an aqueous system to the original formulation of Roundup were compared. Carefully designed and un-confounded laboratory toxicity tests are crucial for accurate assessment of potential risks from the original formulation of Roundup to North American amphibians in aquatic environments. The formulated mixture of this herbicide as well as its components, isopropylamine (IPA) salt of glyphosate and the surfactant MON 0818 (containing polyethoxylated tallowamine (POEA)) were separately tested in 96 h acute toxicity tests with Gosner stage 25 larval anurans. Rana pipiens, R. clamitans, R. catesbeiana, Bufo fowleri, and Hyla chrysoscelis were reared from egg masses and exposed to a series of 11 concentrations of the original formulation of Roundup herbicide, nine concentrations of MON 0818 and three concentrations of IPA salt of glyphosate in static (non-renewal) aqueous laboratory tests. LC50 values are expressed as glyphosate acid equivalents (ae) or as mg/L for MON 0818 concentrations for comparison between the formulation and components. R. pipiens was the most sensitive of five species with 96 h-LC50 values for formulation tests, for the five species, ranging from 1.80 to 4.22 mg ae/L, and MON 0818 exposures with 96 h-LC50 values ranging from 0.68 to 1.32 mg/L. No significant mortality was observed during exposures of 96 h for any of the five species exposed to glyphosate IPA salt at concentrations up to 100 times the predicted environmental concentration (PEC). These results agree with previous studies which have noted that the surfactant MON 0818 containing POEA contributes the majority of the toxicity to the herbicide formulations for fish, aquatic invertebrates, and amphibians. These study results suggest that anurans are among the most sensitive species, and emphasize the importance of testing the herbicide formulation in addition to its separate components to accurately characterize the toxicity and potential risk of the formulation.

http://www.ncbi.nlm.nih.gov/pubmed/22137360

Vários estudos, como os seguintes, mostram que herbicidas à base de glifosato tendem a ser altamente persistentes no solo, na água e no ar, penetrando cadeias e agravando problemas de contaminação ambiental.

Kjaer, J.; Olsen, P.; Ullum, M.; Grant, R. 2005. Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. *J Environ Qual.*, 34(2):608-20.

Pesticide leaching is an important process with respect to contamination risk to the aquatic environment. The risk of leaching was thus evaluated for glyphosate (N-phosphonomethyl-glycine) and its degradation product AMPA (amino-methylphosphonic acid) under field conditions at one sandy and two loamy sites. Over a 2-yr period, tile-drainage water, ground water, and soil water were sampled and analyzed for pesticides. At a sandy site, the strong soil sorption capacity and lack of macropores seemed to prevent leaching of both glyphosate and AMPA. At one loamy site, which received low precipitation with little intensity, the residence time within the root zone seemed sufficient to prevent leaching of glyphosate, probably due to degradation and sorption. Minor leaching of AMPA was observed at this site, although the concentration was generally low, being on the order of 0.05 microg L(-1) or less. At another loamy site, however, glyphosate and AMPA leached from the root zone into the tile drains (1 m below ground surface [BGS]) in average concentrations exceeding 0.1 microg L(-1), which is the EU threshold value for drinking water. The leaching of glyphosate was mainly governed by pronounced macropore flow occurring within the first months after application. AMPA was frequently detected more than 1.5 yr after application, thus indicating a minor release and limited degradation capacity within the soil. Leaching has so far been confined to the depth of the tile drains, and the pesticides have rarely been detected in monitoring screens located at lower depths. This study suggests that as both glyphosate and AMPA can leach through structured soils, they thereby pose a potential risk to the aquatic environment.

http://www.ncbi.nlm.nih.gov/pubmed/15758114

Peruzzo, P.; Porta, A.; Ronco, A. 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. *Environmental Pollution*, 156, 61-66.

Levels of glyphosate were determined in water, soil and sediment samples from a transgenic soybean cultivation area located near to tributaries streams of the Pergamino-Arrecifes system in the north of the Province of Buenos Aires, Argentina. Field work took into account both the pesticide application and the rains occurring after applications. The pesticide was analysed by HPLC-UV detection, previous derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl). In addition, SoilFug multimedia model was used to analyse the environmental distribution of the pesticides. In the field, levels of glyphosate in waters ranged from 0.10 to 0.70 mg/L, while in sediments and soils values were between 0.5 and 5.0 mg/Kg. Temporal variation of glyphosate levels depended directly on the time of application and the rain events. The results obtained from the application of the model are in accordance with the values found in the field.

http://www.ncbi.nlm.nih.gov/pubmed/18308436

Borggaard, O.; Gimsing, A. 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag Sci*, 64(4):441-56.

The very wide use of glyphosate to control weeds in agricultural, silvicultural and urban areas throughout the world requires that special attention be paid to its possible transport from terrestrial to aquatic environments. The aim of this review is to present and discuss the state of knowledge on sorption, degradation and leachability of glyphosate in soils. Difficulties of drawing clear and unambiguous conclusions because of strong soil dependency and limited conclusive investigations are pointed out. Nevertheless, the risk of ground and surface water pollution by glyphosate seems limited because of sorption onto variable-charge soil minerals, e.g. aluminium and iron oxides, and because of microbial degradation. Although sorption and degradation are affected by many factors that might be expected to affect glyphosate mobility in soils, glyphosate leaching seems mainly determined by soil structure and rainfall. Limited leaching has been observed in non-structured sandy soils, while subsurface leaching to drainage systems was observed in a structured soil with preferential flow in macropores, but only when high rainfall followed glyphosate application. Glyphosate in drainage water runs into surface waters but not necessarily to groundwater because it may be sorbed and degraded in deeper soil layers before reaching the groundwater. Although the transport of glyphosate from land to water environments seems very limited, knowledge about subsurface leaching and surface runoff of glyphosate as well as the importance of this transport as related to ground and surface water quality is scarce.

http://www.ncbi.nlm.nih.gov/pubmed/18161065

Chang, F.; Simci, M.; Capel, P. 2011. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem*, 30(3):548-55. doi: 10.1002/etc.431.

This is the first report on the ambient levels of glyphosate, the most widely used herbicide in the United States, and its major degradation product, aminomethylphosphonic acid (AMPA), in air and rain. Concurrent, weekly integrated air particle and rain samples were collected during two growing seasons in agricultural areas in Mississippi and Iowa. Rain was also collected in Indiana in a preliminary phase of the study. The frequency of glyphosate detection ranged from 60 to 100% in both air and rain. The concentrations of glyphosate ranged from <0.01 to 9.1 ng/m(3) and from <0.1 to 2.5 μg/L in air and rain samples, respectively. The frequency of detection and median and maximum concentrations of glyphosate in air were similar or greater to those of the other high-use herbicides observed in the Mississippi River basin, whereas its concentration in rain was greater than the other herbicides. It is not known what percentage of the applied glyphosate is introduced into the air, but it was estimated that up to 0.7% of application is removed from the air in rainfall. Glyphosate is efficiently removed from the air; it is estimated that an average of 97% of the glyphosate in the air is removed by a weekly rainfall ≥ 30 mm.

http://www.ncbi.nlm.nih.gov/pubmed/21128261

Coupe, R.; Kalkhoff, S.; Capel, P.; Gregoire, C. 2011. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Manag Sci.*, 68(1): 16–30.

Background: Glyphosate [N-(phosphonomethyl)glycine] is a herbicide used widely throughout the world in the production of many crops and is heavily used on soybeans, corn and cotton. Glyphosate is used in almost all agricultural areas of the United States, and the agricultural use of glyphosate has increased from less than 10 000 Mg in 1992 to more than 80 000 Mg in 2007. The greatest intensity of glyphosate use is in the midwestern United States, where applications are predominantly to genetically modified corn and soybeans. In spite of the increase in usage across the United States, the characterization of the transport of glyphosate and its degradate aminomethylphosphonic acid (AMPA) on a watershed scale is lacking.
Results: Glyphosate and AMPA were frequently detected in the surface waters of four agricultural basins. The frequency and magnitude of detections varied across basins, and the load, as a percentage of use, ranged from 0.009 to 0.86% and could be related to three general characteristics: source strength, rainfall runoff and flow route.
Conclusions: Glyphosate use in a watershed results in some occurrence in surface water; however, the watersheds most at risk for the offsite transport of glyphosate are those with high application rates, rainfall that results in overland runoff and a flow route that does not include transport through the soil.

http://www.ncbi.nlm.nih.gov/pubmed/21681915

Aparicio, V.; De Gerónimo, E.; Marino, D.; Primost, J.; Carriquiriborde, P.; Costa, J. 2013. Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. *Chemosphere,* 93(9):1866-73. doi: 10.1016/j.chemosphere.2013.06.041.

Argentinian agricultural production is fundamentally based on a technological package that combines no-till and glyphosate in the cultivation of transgenic crops. Transgenic crops (soybean, maize and cotton) occupy 23 million hectares. This means that glyphosate is the most employed herbicide in the country, where 180-200 million liters are applied every year. The aim of this work is to study the environmental fate of glyphosate and its major degradation product, aminomethylphosphonic acid (AMPA), in surface water and soil of agricultural basins. Sixteen agricultural sites and forty-four streams in the agricultural basins were sampled three times during 2012. The samples were analyzed by UPLC-MS/MS ESI(+/-). In cultivated soils, glyphosate was detected in concentrations between 35 and 1502 μg kg(-1), while AMPA concentration ranged from 299 to 2256 μg kg(-1). In the surface water studied, the presence of glyphosate and AMPA was detected in about 15% and 12% of the samples analyzed, respectively. In suspended particulate matter, glyphosate was found in 67% while AMPA was present in 20% of the samples. In streams sediment glyphosate and AMPA were also detected in 66% and 88.5% of the samples respectively. This study is, to our knowledge, the first dealing with glyphosate fate in agricultural soils in Argentina. In the present study, it was demonstrated that glyphosate and AMPA are present in soils under agricultural activity. It was also found that in stream samples the presence of glyphosate and AMPA is relatively more frequent in suspended particulate matter and sediment than in water.

http://www.ncbi.nlm.nih.gov/pubmed/23849835

Battaglin, W.; Meyer, M.; Kuivila, K.; Dietze, J. 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. *JAWRA Journal of the American Water Resources Association,* 50:10.1111/jawr.2014.50.issue-2, 275-290.

Glyphosate use in the United States increased from less than 5,000 to more than 80,000 metric tons/yr between 1987 and 2007. Glyphosate is popular due to its ease of use on soybean, cotton, and corn crops that are genetically modified to tolerate it, utility in no-till farming practices, utility in urban areas, and the perception that it has low toxicity and little mobility in the environment. This compilation is the largest and most comprehensive assessment of the environmental occurrence of glyphosate and aminomethylphosphonic acid (AMPA) in the United States conducted to date, summarizing the results of 3,732 water and sediment and 1,018 quality assurance samples collected between 2001 and 2010 from 38 states. Results indicate that glyphosate and AMPA are usually detected together, mobile, and occur widely in the environment. Glyphosate was detected without AMPA in only 2.3% of samples, whereas AMPA was detected without glyphosate in 17.9% of samples. Glyphosate and AMPA were detected frequently in soils and sediment, ditches and drains, precipitation, rivers, and streams; and less frequently in lakes, ponds, and wetlands; soil water; and groundwater. Concentrations of glyphosate were below the levels of concern for humans or wildlife; however, pesticides are often detected in mixtures. Ecosystem effects of chronic low-level exposures to pesticide mixtures are uncertain. The environmental health risk of low-level detections of glyphosate, AMPA, and associated adjuvants and mixtures remain to be determined.

http://onlinelibrary.wiley.com/doi/10.1111/jawr.12159/abstract

Majewski, M.; Coupe, R.; Foreman, W.; Capel, P. 2014. Pesticides in Mississippi air and rain: a comparison between 1995 and 2007. *Environmental Toxicology and Chemistry*, 33(6):1283-1293.

A variety of current-use pesticides were determined in weekly composite air and rain samples collected during the 1995 and 2007 growing seasons in the Mississippi Delta (MS, USA) agricultural region. Similar sampling and analytical methods allowed for direct comparison of results. Decreased overall pesticide use in 2007 relative to 1995 generally resulted in decreased detection frequencies in air and rain; observed concentration ranges were similar between years, however, even though the 1995 sampling site was 500 m from active fields whereas the 2007 sampling site was within 3 m of a field. Mean concentrations of detections were sometimes greater in 2007 than in 1995, but the median values were often lower. Seven compounds in 1995 and 5 in 2007 were detected in ≥50% of both air and rain samples. Atrazine, metolachlor, and propanil were detected in ≥50% of the air and rain samples in both years. Glyphosate and its degradation product, aminomethyl-phosphonic acid (AMPA), were detected in ≥75% of air and rain samples in 2007 but were not measured in 1995. The 1995 seasonal wet depositional flux was dominated by methyl parathion (88%) and was >4.5 times the 2007 flux. Total herbicide flux in 2007 was slightly greater than in 1995 and was dominated by glyphosate. Malathion, methyl parathion, and degradation products made up most of the 2007 nonherbicide flux.

http://www.ncbi.nlm.nih.gov/pubmed/24549493

Mercurio, P.; Flores, F.; Mueller, J.; Carter, S.; Negri, A. 2014. Glyphosate persistence in seawater. *Marine Pollution Bulletin*, 85(2): 385-390.

Glyphosate is one of the most widely applied herbicides globally but its persistence in seawater has not been reported. Here we quantify the biodegradation of glyphosate using standard "simulation" flask tests with native bacterial populations and coastal seawater from the Great Barrier Reef. The half-life for glyphosate at 25 °C in low-light was 47 days, extending to 267 days in the dark at 25 °C and 315 days in the dark at 31 °C, which is the longest persistence reported for this herbicide. AMPA, the microbial transformation product of glyphosate, was detected under all conditions, confirming that degradation was mediated by the native microbial community. This study demonstrates glyphosate is moderately persistent in the marine water under low light conditions and is highly persistent in the dark. Little degradation would be expected during flood plumes in the tropics, which could potentially deliver dissolved and sediment-bound glyphosate far from shore.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0025326X14000228

2.1.2 Herbicidas à base de glufosinato de amônio

As plantas TH vinculadas ao herbicida glufosinato de amônio (GA) têm sido apresentadas como alternativa de flexibilidade ao controle de plantas tolerantes ao glifosato. Do ponto de vista toxicológico, os problemas se assemelham, embora o acúmulo de informações se faça menor no caso deste último.

Os estudos indexados a seguir apontam problemas causados pelo GA para comunidades do solo e de ambientes aquáticos, alterando relações populacionais e provocando desequilíbrios ambientais, entre outros.

Ahmad, I.; Malloch, D. 1995. Interaction of soil microflora with the bioherbicide phosphinothricin. *Agriculture, Ecosystems & Environment*, Volume 54, Issue 3, July 1995, Pages 165–174.

Phosphinothricin, a microbial toxin synthesized industrially for chemical weed control and currently under development as a selective weed killer in cultivation of transgenic plants engineered to resist its presence, is investigated for its effects on the distribution of microorganisms in 15 agricultural and non-agricultural soils. In agricultural soils, the presence of 1 mM phosphinothricin reduced the number of fungi isolated by about 20% and bacteria by about 40%. Under these conditions the isolation of bacteria from boreal forest soils was also suppressed by about 20%. Differences in herbicide resistance were confirmed when a random selection of fungi, bacteria and actinomycetes isolated in the absence of herbicide was grown with 1 mM phosphinothricin. Soil isolates growing in the presence of 1 mM phosphinothricin exhibited a wide spectrum of tolerance to increasing herbicide concentration over the range of 0–50 mM phosphinothricin. Of fungal isolates, the plant pathogen *Verticilium alboatrum* was among the most resistant, while the mycoparasitic species *Trichoderma harzianum* and *T. longipilus* were among the most sensitive to the presence of phosphinothricin.

http://www.sciencedirect.com/science/article/pii/016788099500603P

Ahmad, I.; Bissett, J.; Malloch, D. 1995. Effect of phosphinothricin on nitrogen metabolism of *Trichoderma* species and its implications for their control of phytopathogenic fungi. *Pesticide Biochemistry and Physiology*, Volume 53, Issue 1, Pages 49–59.

The antagonistic control of the phytopathogen *Fusarium oxysporum* by *Trichoderma* species is impaired in the presence of phosphinothricin, a microbial toxin commercialized for chemical weed control under trade names Basta, Ignite, and Herbiace. The influence of phosphinothricin on growth parameters and nitrogen metabolism in nine strains of *Trichoderma* and a closely related fungus *Nectria ochroleuca* was investigated. The presence of 1 m*M* phosphinothricin was lethal to the *Trichoderma* anamorph of *Hypocrea gelatinosa* and almost equally inhibitory to *Trichoderma polysporum*. In other species it caused marked reductions in the hyphal protein content, accompanied by lower biomass yields in all except *N. ochroleuca*. Under normal growth conditions, these rapidly growing fungi maintain highly active levels of the two key enzymes of ammonium assimilation, glutamine synthetase, and NADPH-dependent glutamate dehydrogenase. Phosphinothricin, by causing an inhibition in glutamine synthetase activity, diminishes the enzymic potential for the combined operation of these two alternative pathways. The level of NADPH-glutamate dehydrogenase activity was exceptionally high in *N. ochroleuca,* and although declining in the presence of phosphinothricin, remained much higher than those in nine strains of *Trichoderma*. The levels of NADPH-glutamate dehydrogenase in *T. harzianum, T. citrinoviride,* and *T. viride* were higher in the presence than in the absence of phosphinothricin. In the presence of phosphinothricin, the levels of both aspartate aminotransferase and alanine aminotransferase activities were stimulated in *T. harzianum, T. atroviride, T. citrinoviride,* and *T. viride* and that of alanine aminotransferase in *T. koningii*. *Trichoderma harzianum* and *T. atroviride* contain single anionic molecular forms of glutamine synthetase, NADPH-glutamate dehydrogenase, and alanine aminotransferase and two isoforms of aspartate aminotransferase both in the presence and in the absence of phosphinothricin.

http://www.sciencedirect.com/science/article/pii/S0048357585710541

De Snoo, G.; De Jong, F.; Der Poll, R.; Van Der Linden, M. 2001. Effects of glufosinate ammonium on off crop vegetation--interim results. *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet*, 66(2b): 731-41.

In 2000 a field study was conducted at four different locations concerning the effects of low dosages of glufosinate-ammonium, a leaf acting herbicide, on off crop vegetation. Therefore species rich road verges and ditch banks not adapted to a history of herbicide use were sprayed twice with different dosages of glufosinate-ammonium, simulating drift (0, 2, 4, 16, 32 and 64% of the maximum field dose: 800 g a.i./ha). The parameters studied were short term phytotoxic effects and the effects on biomass, species cover and number of species in autumn (Braun-Blanquêt relevés). The results show significant phytotoxic effects at all dosages of glufosinate-ammonium on the non-target vegetation. The low concentrations (2 and 4%) had most impact when applied early in the season (9% average at the 2% dosage and 22% at 4% dosage, after the first spraying. At high dosages (32 and 64%) a decrease of the biomass of the vegetation was found in August. A comparison between treatments in August shows a small decrease in species number and cover in the 64% compared to the control. In the comparison between the spring and August relevés, the decrease in the mean number of species was significantly stronger in the treated plots than in the untreated ones of 4% and higher. For monocotyledons in all treatments except 16%, a significantly stronger decrease in species number was found compared to the untreated. For dicotyledons only the 64% dosage differed from the untreated. Only at the 64% treatment the total cover of species decreased more than in the untreated plots. Since drift percentages of 2-4% can be expected at 1-2 m from a treated plot it can be concluded that the use of glufosinate-ammonium could result in visible short term phytotoxic effects (max 22%) on off-crop vegetation such as ditch banks and verges. There are also indications that effects on the number and cover of species in autumn can occur. Because in future glufosinate-ammonium could be used on a large scale in herbicide resistant crops like maize, which will be cultivated on the same field for many years, this study will be continued in 2001 in order to investigate if there are--whether or not--sustainable effects on the off crop vegetation.

http://www.ncbi.nlm.nih.gov/pubmed/12425097

Carpenter, D.; Boutin, C. 2010. Sublethal effects of the herbicide glufosinate ammonium on crops and wild plants: short-term effects compared to vegetative recovery and plant reproduction. *Ecotoxicology*, 19: 1322-1326.

Current guidelines for phytotoxicity testing rely heavily on short-term testing of primarily crop species to predict the sensitivity of non-target, wild plants to herbicides. However, little is known on how plants recover following initial growth inhibitions in standard 14-28 day greenhouse tests conducted for pesticide assessment and registration. The objectives of this study were to assess the ability of plant species to recover (biomass and reproduction) when tested at the juvenile stage (routine regulatory testing), comparing crop and wild species and using the herbicide glufosinate ammonium. Ten crops and 10 wild species were tested with a one-time exposure to glufosinate ammonium in a greenhouse. Half the plants of each species (9 doses × 6 replicates) were harvested 3 weeks after being sprayed (short-term). The remaining plants were harvested several weeks later, coinciding with seed set or natural senescence (long-term). Total aboveground biomass and several endpoints related to crop production and plant reproduction were measured. Calculated IC50 values (dosage that results in a 50% decrease in the biomass of a plant as compared to the untreated controls) based solely on aboveground biomass, for species harvested in the long-term were generally higher than those obtained in the short-term (with two exceptions), indicating recovery over time. Crop species did not differ from wild species in terms of sensitivity. However, in seven out of 12 cases where reproduction was measurable, reproductive endpoints were more sensitive than either short or long-term biomass endpoints, indicating the importance of examining these parameters in phytotoxicity testing. Glufosinate ammonium was found to be phytotoxic at low doses (2.64-7.74% g ai/ha of the label rate).

http://www.ncbi.nlm.nih.gov/pubmed/20635139

2.1.3 Herbicidas à base de 2,4-D

A emergência de plantas tolerantes ao glifosato (e, em menor grau, ao glufosinato de amônia) abriu oportunidade de mercado a novas tecnologias na medida em que se reduzia a eficácia daquelas PGMs tornando mais complexo o manejo de plantas adventícias, em áreas de lavoura TH. A disponibilidade de estoques globais do herbicida 2,4-D, proibições de seu uso em vários países, o fato de se tratar de tecnologia dominada e sob domínio público acabaram por colocá-lo como alternativa interessante para o agronegócio, em que pesem os riscos alarmantes para a saúde humana, animal e ambiental.

No momento em que se concluía essa publicação, a CTNBio autorizou plantio e consumo da primeira variedade de soja tolerante ao 2,4-D, sendo de esperar que essa tecnologia substitua variedades tolerantes ao glifosato e/ou ao glufosinato de amônio. Eventos de milho com essa mesma função constam também na pauta do órgão, e é de aguardar sua liberação comercial para os próximos meses.Trata-se de "solução" temporária, consequência lógica da expansão de PGMs TH. É possível afirmar, desde agora, que o uso massivo de 2,4-D em poucos anos determinará maior surgimento de populações de plantas adventícias insensíveis àquele princípio ativo – populações que já existem esporadicamente ao redor do mundo, em especial nos locais onde há uso importante daquele herbicida.

Em outras palavras, as PGMs tolerantes aos herbicidas à base de 2,4-D surgem como tentativa de protelar crises de gestão de lavouras ocupadas por plantas ruderais insensíveis ao glifosato, entre outros. Isso se verifica de forma especialmente aguda nas lavouras de milho, algodão e soja, e sua adoção deve ocorrer por parte significativa dos agricultores confrontados com tais problemas de manejo. Consequentemente, pode-se prever um aumento drástico no uso desse herbicida ao longo dos próximos anos[47].

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[47] Eventos de plantas tolerantes aos herbicidas à base de 2,4-D ainda não são cultivadas/comercializadas, mas já foram liberadas para plantio em três país no mundo, o Canadá em 2012, os EUA em 2014 e o Brasil em março de 2015.

Parte das implicações ambientais decorrentes dessa expansão no uso do 2,4-D será discutida a seguir.

Roberts, B.; Dorough, H. 1984. Relative toxicity of chemical to the earthworm *Eisenia foetida*. *Environmental Toxicology and Chemistry*, 3: 67-78.

In the present study, 90 chemicals were tested against *Eisenia foetida* for the purpose of using this organism as the marker species to indicate the relative toxicities of chemicals to earthworms and other soil invertebrates. The worms were exposed to deposits of the chemicals on filter paper for 48 h and the mortality was recorded; concentrations were expressed in µg/cm^2. Based on the resulting LC_{50} values, the chemicals were classified as supertoxic (< 1.0 µg/cm^2), extremely toxic (1–10 µg/cm^2), very toxic (10–100 µg/cm^2), moderately toxic (100–1,000 µg/cm^2) or relatively nontoxic (> 1,000 µg/cm^2). Of the chemicals tested (pesticides, solvents, metals, drugs, carcinogens, etc.), only carbofuran and eserine salicylate, both carbamates, were supertoxic. The remaining chemicals were distributed about equally among the other toxicity categories. The most surprising results were that the phenolic hydrolytic products of parathion, carbaryl, 2,4-D and 2,4,5-T were as toxic, or more toxic, than the parent material, with all of these compounds falling within the extremely toxic and very toxic classifications. Several chemicals, considered only moderately or relatively nontoxic to mammals, were extremely or very toxic to earthworms; among these compounds were carbaryl, malathion, cypermethrin and benomyl. The results of this study further demonstrate the unpredictability of chemical toxicity to different animal species, a fact which complicates the assessment of environmental risk to one or more species based on data attained with another.

http://onlinelibrary.wiley.com/doi/10.1002/etc.5620030109/abstract

Arias, E. 2003. Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. *Ecotoxicol Environ Saf*, 55(3): 338-43.

As genetic damage may result from exposure to agricultural chemicals, it seemed appropriate to assess the genotoxic potential of 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used broad-leaf herbicide, using a test system that may provide some indications on the genetic risk to animal species in the wild. In the present study, sister chromatid exchange (SCE) induction and cell cycle kinetics alterations by 2,4-D in 4-day old chick embryos were evaluated. Both a commercial herbicide formulation containing 37% 2,4-D isooctyl ester as active ingredient and pure 2,4-D were tested. Chick embryos were treated with 0, 0.5, 1, 2, or 4 mg 2,4-D. Test solutions were applied to the inner shell membrane on day 0 of incubation. Either commercial formulation or pure 2,4-D induced a dose-related increase in SCE frequency over the concentration range from 0 to 4 mg/embryo. Significantly higher SCE frequency was seen for the 4-mg group of embryos treated with the commercial product. A slightly higher SCE value was observed for the vehicle group (acetone-treated embryos) compared with the negative controls (untreated embryos). Significant inhibition of cell cycle progression was evident in both experimental groups and was generally dose related. The extent of changes in cell kinetics was similar in both groups, although somewhat more marked in the group treated with pure 2,4-D. The present findings corroborate the positive results from recent in vivo rodent studies.

http://www.ncbi.nlm.nih.gov/pubmed/12798768

LaChapelle, A.; Ruygrok, M.; Toomer, M.; Oost, J.; Monnie, M.; Swenson, J.; Compton, A.; Stebbins-Boaz, B. 2007. The hormonal herbicide, 2,4-dichlorophenoxyacetic acid, inhibits *Xenopus oocyte* maturation by targeting translational and posttranslational mechanisms. *Reproductive toxicology*, 23(1):20-31.

The widely used hormonal herbicide, 2,4-dichlorophenoxyacetic acid, blocks meiotic maturation in vitro and is thus a potential environmental endocrine disruptor with early reproductive effects. To test whether maturation inhibition was dependent on protein kinase A, an endogenous maturation inhibitor, oocytes were microinjected with PKI, a specific PKA inhibitor, and exposed to 2,4-D. Oocytes failed to mature, suggesting that 2,4-D is not dependent on PKA activity and likely acts on a downstream target, such as Mos. De novo synthesis of Mos, which is triggered by mRNA poly(A) elongation, was examined. Oocytes were microinjected with radiolabelled in vitro transcripts of Mos RNA and exposed to progesterone and 2,4-D. RNA analysis showed progesterone-induced polyadenylation as expected but none with 2,4-D. 2,4-D-activated MAPK was determined to be cytoplasmic in localization studies but poorly induced Rsk2 phosphorylation and activation. In addition to inhibition of the G2/M transition, 2,4-D caused abrupt reduction of H1 kinase activity in MII phase oocytes. Attempts to rescue maturation in oocytes transiently exposed to 2,4-D failed, suggesting that 2,4-D induces irreversible dysfunction of the meiotic signaling mechanism.

http://www.ncbi.nlm.nih.gov/pubmed/17055699

Correia, F.; Moreira, J. 2010. Effects of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) in laboratory tests. *Bull Environ Contam Toxicol*, 85(3):264-8. doi: 10.1007/s00128-010-0089-7.

Laboratory tests were conducted to compare the effects of various concentrations of glyphosate and 2,4-D on earthworms (Eisenia foetida) cultured in Argissol during 56 days of incubation. The effects on earthworm growth, survival, and reproduction rates were verified for different exposure times. Earthworms kept in glyphosate-treated soil were classified as alive in all evaluations, but showed gradual and significant reduction in mean weight (50%) at all test concentrations. For 2,4-D, 100% mortality was observed in soil treated with 500 and 1,000 mg/kg. At 14 days, 30%-40% mortality levels were observed in all other concentrations. No cocoons or juveniles were found in soil treated with either herbicide. Glyphosate and 2,4-D demonstrated severe effects on the development and reproduction of Eisenia foetida in laboratory tests in the range of test concentrations.

http://www.ncbi.nlm.nih.gov/pubmed/20658223

No caso dos herbicidas à base de 2,4-D (e também de glufosinato de amônio, ainda que em menor escala) cabe especial atenção ao fato de que tais substâncias formam microgotas com alto potencial de deriva[48]. Nessas situações, as substâncias voláteis circulam ao sabor da umidade do ar e dos ventos, alcançando longas distâncias e "queimando" as plantas susceptíveis que eventualmente alcancem. Espécies agrícolas ou selvagens, de pequeno ou grande porte, se farão ameaçadas de forma dramática, com as implicações socioeconômicas previsíveis.

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[48] Impactos negativos associados ao potencial de deriva possuem duas causas potenciais: a deriva aerotransportada (ou de gota) – quando o produto se move para fora do alvo durante a aplicação – e a deriva de vapor – quando o produto se move para fora do alvo após a aplicação – e que caracteriza um produto volátil. Em função da formulação do herbicida comercial, o 2,4-D pode ser altamente volátil, em especial quando inclua sais e ésteres. Nas suas outras formulações (ácidos, por exemplo), a maior parte dos impactos causados pela deriva será devida à deriva aerotransportada, podendo gerar danos importantes em lavouras vizinhas. Cabe destacar que o 2,4-D apresenta riscos diferenciados em relação aos impactos potenciais de deriva principalmente por causa da sua alta fitotoxicidade em doses extremamente baixas.

Sciumbato, A.; Chandler, J.; Senseman, S.; Bovey, R.; Smith, K. 2004. Determining exposure to auxin-like herbicides. I. Quantifying injury to cotton and soybean. *Weed Technology*, 18: 1125–1134.

Volatility and drift are problems commonly associated with auxin-like herbicides. Field and greenhouse studies were conducted at Texas A & M University to develop a method of quantifying volatility and subsequent off-target movement of 2,4-D, dicamba, and triclopyr. Rate–response curves were established by applying reduced rates ranging from 4×10^{-1} to 1×10^{-5} times the normal use rates of the herbicides to cotton and soybean and recording injury for 14 d after treatment (DAT) using a rating scale designed to quantify auxin-like herbicide injury. Injury from herbicide volatility was then produced on additional cotton and soybean plants through exposure to vapors of the dimethylamine salt of 2,4-D, diglycolamine salt of dicamba, and butoxyethyl ester of triclopyr using air chambers inside a greenhouse and volatility plots in the field. Injury resulting from this exposure was evaluated for 14 d using the same injury-evaluation scale that was used to produce the rate–response curves. Volatility-injury data were then applied to the rate–response curves so that herbicide rates corresponding with observed injury could be calculated. Using this method, herbicide volatility rates estimated from greenhouse-cotton injury were determined to be 3.0×10^{-3}, 1.0×10^{-3}, and 4.9×10^{-2} times the use rates of 2,4-D, dicamba, and triclopyr, respectively. Greenhouse-grown soybean developed injury consistent with 1.4×10^{-2}, 1.0×10^{-3}, and 2.5×10^{-2} times the normal use rate of 2,4-D, dicamba, and triclopyr, respectively. Under field conditions, cotton developed injury symptoms that were consistent with 4.0×10^{-3}, 2.0×10^{-3}, and 1.25×10^{-1} times the recommended use rates of 2,4-D, dicamba, and triclopyr, respectively. Field soybean displayed injury symptomology concordant with 1.6×10^{-1}, 1.0×10^{-2}, and 1.1×10^{-1} times the normal use rates of 2,4-D, dicamba, and triclopyr, respectively. This procedure provided herbicide volatility rate estimates that were consistent with rates and injury from the rate–response injury curves. Additional research is needed to ascertain its usefulness in determining long-term effects of drift injury on crop variables such as yield.

http://www.bioone.org/doi/abs/10.1614/WT-03-105R1

2.2 Impactos negativos das lavouras de plantas TH sobre a biodiversidade

A ecotoxicidade dos herbicidas associados aos cultivos de plantas TH determina que os impactos ambientais dessas lavouras sejam relevantes. Além da toxicidade direta, as alterações provocadas por aqueles produtos tendem a desestabilizar cadeias tróficas e ecossistêmicas (a supressão de grande parte da biomassa vegetal ou a desaparição de espécies vegetais fundamentais para a realização de parte dos ciclos biológicos de determinadas espécies de animais, insetos ou micro-organismos) e podem gerar impactos negativos sobre organismos que não são diretamente sensíveis àqueles produtos.

Num agrossistema cultivado com tecnologia TH, plantas ruderais que não reduzem significativamente a produtividade da lavoura podem ser consideradas como organismos não alvo (ONAs), assim como os organismos vegetais que se desenvolvem ao redor das lavouras (APP, RL e qualquer ambiente localizado entre os cultivos – biótipos reconhecidos em seu papel de refúgio para as espécies acossadas em áreas agrícolas, que deles necessitam para manter funções ecossistêmicas). Os artigos abaixo listados nesta parte do livro examinam esses aspectos, concentrando atenção sobre as lavouras tolerantes a glifosato.

Além disto, a biota do solo, e em especial os fungos e bactérias, pode ser negativamente impactada pelo uso dos herbicidas sistematicamente associados ao plantio de variedades TH. Esse tema também é discutido em algumas das referências expostas a seguir. Outras pesquisas, disponíveis no item 3.2.1 da Parte 2, examinam relações entre a degradação da biota do solo em agroecossistemas e os problemas agrícolas (e socioeconômicos) associados.

2.2.1 Animais afetados pelas tecnologias de PGMs TH

Os artigos a seguir discutem impactos da tecnologia sobre animais, aqui considerados como casos especiais de organismos não alvo, também afetados pelas lavouras de PGMs TH.

Watkinson, A.; Freckleton, R.; Robinson, R.; Sutherland, W. 2000. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science*, 289:1554-1557.

We simulated the effects of the introduction of genetically modified herbicide-tolerant (GMHT) crops on weed populations and the consequences for seed-eating birds. We predict that weed populations might be reduced to low levels or practically eradicated, depending on the exact form of management. Consequent effects on the local use of fields by birds might be severe, because such reductions represent a major loss of food resources. The regional impacts of GMHT crops are shown to depend on whether the adoption of GMHT crops by farmers covaries with current weed levels.

http://www.sciencemag.org/content/289/5484/1554

Hawes, C.; Haughton, A.; Osborne, J.; Roy, D.; Clark, S.; Perry, J.; Rothery, P.; Bohan, D.; Brooks, D.; Champion, G.; Dewar, A.; Heard, M.; Woiwod, I.; Daniels, R.; Yound, M.; Parish, A.; Scott, R.; Firbank, L.; Squire, G. 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the farm scale evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London*, B 358, 1899-1913.

Effects of genetically modified herbicide-tolerant (GMHT) and conventional crop management on invertebrate trophic groups (herbivores, detritivores, pollinators, predators and parasitoids) were compared in beet, maize and spring oilseed rape sites throughout the UK. These trophic groups were influenced by season, crop species and GMHT management. Many groups increased twofold to fivefold in abundance between early and late summer, and differed up to 10-fold between crop species. GMHT management superimposed relatively small (less than twofold), but consistent, shifts in plant and insect abundance, the extent and direction of these effects being dependent on the relative efficacies of comparable conventional herbicide regimes. In general, the biomass of weeds was reduced under GMHT management in beet and spring oilseed rape and increased in maize compared with conventional treatments. This change in resource availability had knock-on effects on higher trophic levels except in spring oilseed rape where herbivore resource was greatest. Herbivores, pollinators and natural enemies changed in abundance in the same directions as their resources, and detritivores increased in abundance under GMHT management across all crops. The result of the later herbicide application in GMHT treatments was a shift in resource from the herbivore food web to the detritivore food web. The Farm Scale Evaluations have demonstrated over 3 years and throughout the UK that herbivores, detritivores and many of their predators and parasitoids in arable systems are sensitive to the changes in weed communities that result from the introduction of new herbicide regimes.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1693274/pdf/14561321.pdf.

Brooks, D.; Bohan, D.; Champion, G.; Haughton, A.; Hawes, C.; Heard, M.; Clark, S.; Dewar, A.; Firbank, L.; Perry, J.; Rothery, P.; Scott, R.; Woiwod, I.; Birchall, C.; Skellern, M.; Walker, J.; Baker, P.; Bell, D.; Browne, E.; Dewar, A.; Fairfax, C.; Garner, B.; Haylock, L.; Horne, S.; Hulmes, S.; Mason, N.; Norton, L.; Nuttall, P.; Randle, Z.; Rossall, M.; Sands, R.; Singer, E.; Walker, M. 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. *Philosophical Transactions of the Royal Society of London*, B 358, 1847-1862.

The effects of herbicide management of genetically modified herbicide-tolerant (GMHT) beet, maize and spring oilseed rape on the abundance and diversity of soil-surface-active invertebrates were assessed. Most effects did not differ between years, environmental zones or initial seedbanks or between sugar and fodder beet. This suggests that the results may be treated as generally applicable to agricultural situations throughout the UK for these crops. The direction of the effects was evenly balanced between increases and decreases in counts in the GMHT compared with the conventional treatment. Most effects involving a greater capture in the GMHT treatments occurred in maize, whereas most effects involving a smaller capture were in beet and spring oilseed rape. Differences between GMHT and conventional crop herbicide management had a significant effect on the capture of most surface-active invertebrate species and higher taxa tested in at least one crop, and these differences reflected the phenology and ecology of the invertebrates. Counts of carabids that feed on weed seeds were smaller in GMHT beet and spring oilseed rape but larger in GMHT maize. In contrast, collembolan detritivore counts were significantly larger under GMHT crop management.

Artigo completo em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1693272/pdf/14561318.pdf

Heard, M.; Hawes, C.; Champion,G.; Clark, S.; Firbank, L.; Haughton, A.; Parish, A.; Perry, J.; Rothery, P.; Scott, R.; Skellern, M.; Squire, G.; Hill, M. 2003. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. I. Effects on abundance and diversity. *Philos Trans R Soc Lond B Biol Sci*, 29;358 (1439):1819-32.

We compared the seedbanks, seed rains, plant densities and biomasses of weeds under two contrasting systems of management in beet, maize and spring oilseed rape. Weed seedbank and plant density were measured at the same locations in two subsequent seasons. About 60 fields were sown with each crop. Each field was split, one half being sown with a conventional variety managed according to the farmer's normal practice, the other half being sown with a genetically modified herbicide-tolerant (GMHT) variety, with weeds controlled by a broad-spectrum herbicide. In beet and rape, plant densities shortly after sowing were higher in the GMHT treatment. Following weed control in conventional beet, plant densities were approximately one-fifth of those in GMHT beet. In both beet and rape, this effect was reversed after the first application of broad-spectrum herbicide, so that late-season plant densities were lower in the GMHT treatments. Biomass and seed rain in GMHT crops were between one-third and one-sixth of those in conventional treatments. The effects of differing weed-seed returns in these two crops persisted in the seedbank: densities following the GMHT treatment were about 20% lower than those following the conventional treatment. The effect of growing maize was quite different. Weed density was higher throughout the season in the GMHT treatment. Late-season biomass was 82% higher and seed rain was 87% higher than in the conventional treatment. The difference was not subsequently detectable in the seedbank because the total seed return was low after both treatments. In all three crops, weed diversity was little affected by the treatment, except for transient effects immediately following herbicide application.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1693279/

Bohan, D.; Boffey, C.; Brooks, D.; Clark, S.; Dewar, A.; Firbank, L.; Haughton, A.; Hawes, C.; Heard, M.; May, M.; Osborne, J.; Perry, J.; Rothery, P.; Roy, D.; Scott, R.; Squire, G.; Woiwod, I.; Champion, G. 2005. Effects on weed and invertebrate abundance and diversity of herbicide management in genetically modified herbicide-tolerant winter-sown oilseed rape. *Proc Biol Sci*, B Vol. 272 (1562): 463-474.

We evaluated the effects of the herbicide management associated with genetically modified herbicide-tolerant (GMHT) winter oilseed rape (WOSR) on weed and invertebrate abundance and diversity by testing the null hypotheses that there is no difference between the effects of herbicide management of GMHT WOSR and that of comparable conventional varieties. For total weeds, there were few treatment differences between GMHT and conventional cropping, but large and opposite treatment effects were observed for dicots and monocots. In the GMHT treatment, there were fewer dicots and monocots than in conventional crops. At harvest, dicot biomass and seed rain in the GMHT treatment were one-third of that in the conventional, while monocot biomass was threefold greater and monocot seed rain almost fivefold greater in the GMHT treatment than in the conventional. These differential effects persisted into the following two years of the rotation. Bees and Butterflies that forage and select for dicot weeds were less abundant in GMHT WORS management in July. Year totals for Collembola were greater under GMHT management. There were few other treatment effects on invertebrates, despite the marked effects of herbicide management on the weeds.

http://www.ncbi.nlm.nih.gov/pubmed/15799941

Gibbons, D.; Bohan, D.; Rothery, P.; Stuart, R.; Haughton, A.; Scott, R.; Wilson, J.; Perry, J.; Clark, S.; Dawson, R.; Firbank, L. 2006. Weed seed resources for birds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. *Proc Biol Sci*, 7;273 (1596):1921-8.

The UK Farm Scale Evaluations (FSEs) have shown that the use of broad spectrum herbicides on genetically modified herbicide-tolerant (GMHT) crops can have dramatic effects on weed seed production compared to management of conventional varieties. Here, we use FSE data and information on bird diets to determine how GMHT cropping might change the food resources available to farmland birds. More than 60 fields of each of four crops, spring- and winter-sown oilseed rape, beet and maize, were split, one half being sown with a conventional variety, the other with a GMHT variety. Seed rain from weeds known to be important in the diets of 17 granivorous farmland bird species was measured under the two treatments. In beet and spring oilseed rape, rain of weed seeds important in the diets of 16 bird species was significantly reduced in GMHT compared to conventional halves; for no species did it increase. In winter oilseed rape, rain of weed seeds important in the diets of 10 species was significantly reduced in GMHT halves; for only one species did it increase significantly. By contrast, in maize, rain of weed seeds important in the diets of seven species was significantly greater in GMHT halves; for no species was it reduced. Treatment effects for the total weed seed energy available to each bird species were very similar to those for seed rain alone. Measuring the effects on individual bird species was outside the scope of this study. Despite this, these results suggest that should beet, spring and winter rape crops in the UK be largely replaced by GMHT varieties and managed as in the FSEs, this would markedly reduce important food resources for farmland birds, many of which declined during the last quarter of the twentieth century. By contrast, GMHT maize would be beneficial to farmland birds.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1634768/

MOD - Brower, L.; Taylor, O.; Williams, E.; Slayback, D.; Zubieta, R.; Ramirez, M. 2012. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? *Insect Conservation and Diversity*, Vol. 5(2): 95-100.

1. During the 2009–2010 overwintering season and following a 15-year downward trend, the total area in Mexico occupied by the eastern North American population of overwintering monarch butterflies reached an all-time low. Despite an increase, it remained low in 2010–2011.
2. Although the data set is small, the decline in abundance is statistically significant using both linear and exponential regression models.
3. Three factors appear to have contributed to reduce monarch abundance: degradation of the forest in the overwintering areas; the loss of breeding habitat in the United States due to the expansion of GM herbicide-resistant crops, with consequent loss of milkweed host plants, as well as continued land development; and severe weather.
4. This decline calls into question the long-term survival of the monarchs' migratory phenomenon.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1752-4598.2011.00142.x/pdf

Pleasants, J.; Oberhauser, K. 2013. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conservation and Diversity*, Vol. 6(2): 135-144.

1. The size of the Mexican overwintering population of monarch butterflies has decreased over the last decade. Approximately half of these butterflies come from the U.S. Midwest where larvae feed on common milkweed. There has been a large decline in milkweed in agricultural fields in the Midwest over the last decade. This loss is coincident with the increased use of glyphosate herbicide in conjunction with increased planting of genetically modified (GM) glyphosate-tolerant corn (maize) and soybeans (soya).
2. We investigate whether the decline in the size of the overwintering population can be attributed to a decline in monarch production owing to a loss of milkweeds in agricultural fields in the Midwest. We estimate Midwest annual monarch production using data on the number of monarch eggs per milkweed plant for milkweeds in different habitats, the density of milkweeds in different habitats, and the area occupied by those habitats on the landscape.
3. We estimate that there has been a 58% decline in milkweeds on the Midwest landscape and an 81% decline in monarch production in the Midwest from 1999 to 2010. Monarch production in the Midwest each year was positively correlated with the size of the subsequent overwintering population in Mexico. Taken together, these results strongly suggest that a loss of agricultural

milkweeds is a major contributor to the decline in the monarch population.
4. The smaller monarch population size that has become the norm will make the species more vulnerable to other conservation threats.

Artigo completo disponivel em http://www.mlmp.org/results/findings/pleasants_and_oberhauser_2012_milkweed_loss_in_ag_fields.pdf

Zaller, J.; Heigl, F.; Ruess, L.; Grabmaier, A. 2014. Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem. *Scientific Reports*, 9;4:5634.

Herbicides containing glyphosate are widely used in agriculture and private gardens, however, surprisingly little is known on potential side effects on non-target soil organisms. In a greenhouse experiment with white clover we investigated, to what extent a globally-used glyphosate herbicide affects interactions between essential soil organisms such as earthworms and arbuscular mycorrhizal fungi (AMF). We found that herbicides significantly decreased root mycorrhization, soil AMF spore biomass, vesicles and propagules. Herbicide application and earthworms increased soil hyphal biomass and tended to reduce soil water infiltration after a simulated heavy rainfall. Herbicide application in interaction with AMF led to slightly heavier but less active earthworms. Leaching of glyphosate after a simulated rainfall was substantial and altered by earthworms and AMF. These sizeable changes provide impetus for more general attention to side-effects of glyphosate-based herbicides on key soil organisms and their associated ecosystem services.

Artigo completo disponível em http://www.nature.com/srep/2014/140709/srep05634/pdf/srep05634.pdf

2.2.2 Vegetais (não alvo) afetados pelas tecnologias de PGMs TH

Os artigos seguintes analisam impactos de PGMs TH sobre vegetais interpretados como organismos não alvo da tecnologia.

REV - Pengue, W. 2005. Transgenic Crops in Argentina: The Ecological and Social Debt. *Bulletin of Science, Technology & Society*, Vol. 25, No. 4, 314-322.

There is no doubt that soybean is the most important crop for Argentina, with a planted surface that rose 11,000,000 hectares and a production of around 35,000,000 metric tons. During the 1990s, there was a significant agriculture transformation in the country, motorize by the adoption of transgenic crops (soy-bean, maize, and cotton) under the no-tillage system. The expansion of this model has been spread not only in the Pampas but also in very rich areas with high biodiversity, opening a new agricultural border to important eco-regions like the Yungas, Great Chaco, and the Mesopotamian Forest. Transgenic cropping is a powerful technology. This produced relevant transformations over the environment and society where it is allowed. Migration, concentration of agribusiness, and loss of food sovereignty are some of the social results. Landscape transformation in the rural sector is evident, and the appearance of tolerance weeds to glyphosate is a reality. Nutrient depletion, soil-structure degradation, potential desertification, and loss of species are other consequences on the environmental level.

http://bst.sagepub.com/content/25/4/314.abstract

Strandberg, B.; Pedersen, M.; Elmegaard, N. 2005. Weed and arthropod populations in conventional and genetically modified herbicide tolerant fodder beet fields. *Agriculture, Ecosystems and Environment*, 105, 243–253.

The introduction of genetically modified herbicide tolerant (GMHT) crops has raised concerns from both scientists and non-governmental organisations about possible effects on arable flora and fauna due to the changes in herbicide application and management that such crops involve. Three consecutive studies were performed, covering flora and fauna in fields of GMHT and conventional fodder beets over the season, at different locations and under different spraying regimes. At all locations and in the 3 years, a denser and more diverse weed flora and arthropod fauna were found in GMHT beets in early and mid-summer than in conventional beets when glyphosate-treatment occurred at or after label recommendation. Following the herbicide applications the GMHT fields had fewer weed species and seeds and lower weed densities and biomass than conventional fields. However, application of glyphosate earlier than recommended resulted in an extremely low weed diversity, density and biomass during the entire season. Timing of the first glyphosate applications, i.e. the duration of the herbicide free period, was essential in terms of biodiversity improvements. In the long term reduced production of weed seeds in GMHT fields may deplete the weed flora if the GMHT strategy becomes widely adopted.

http://www.sciencedirect.com/science/article/pii/S0167880904001288

Firbank, L.; Rothery, P.; May, M.; Clark, S.; Scott, R.; Stuart, R.; Boffey, C.; Brooks, D.; Champion, G.; Haughton, A.; Hawes, C; Heard, M.; Dewar, A.; Perry, J.; Squire, G. 2006. Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. *Biol Lett*, 2(1): 140–143. doi: 10.1098/rsbl.2005.0390.

The Farm Scale Evaluations (FSEs) showed that genetically modified herbicide-tolerant (GMHT) cropping systems could influence farmland biodiversity because of their effects on weed biomass and seed production. Recently published results for winter oilseed rape showed that a switch to GMHT crops significantly affected weed seedbanks for at least 2 years after the crops were sown, potentially causing longer-term effects on other taxa. Here, we seek evidence for similar medium-term effects on weed seedbanks following spring-sown GMHT crops, using newly available data from the FSEs. Weed seedbanks following GMHT maize were significantly higher than following conventional varieties for both the first and second years, while by contrast, seedbanks following GMHT spring oilseed rape were significantly lower over this period. Seedbanks following GMHT beet were smaller than following conventional crops in the first year after the crops had been sown, but this difference was much reduced by the second year for reasons that are not clear. These new data provide important empirical evidence for longer-term effects of GMHT cropping on farmland biodiversity.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1617187/

Altieri, Miguel. 2009. The ecological impacts of large-scale agrofuel monoculture production systems in the Americas. *Bulletin of Science Technology Society*, vol. 29 no. 3 236-244.

This article examines the expansion of agrofuels in the Americas and the ecological impacts associated with the technologies used in the production of large-scale monocultures of corn and soybeans. In addition to deforestation and displacement of lands devoted to food crops due to expansion of agrofuels, the massive use of transgenic crops and agrochemical inputs, mainly fertilizers and herbicides used in the production of agrofuels, pose grave environmental problems.

http://bst.sagepub.com/content/29/3/236.abstract

REV - Mortensen, D.; Egan, J.; Maxwell, D.; Ryan, M.; Smith, R. 2012. Navigating a critical juncture for sustainable weed management. *BioScience*, Vol. 62, No. 1, pp. 75-84.

Agricultural weed management has become entrenched in a single tactic—herbicide-resistant crops—and needs greater emphasis on integrated practices that are sustainable over the long term. In response to the outbreak of glyphosate-resistant weeds, the seed and agrichemical industries are developing crops that are genetically modified to have combined resistance to glyphosate and synthetic auxin herbicides. This technology will allow these herbicides to be used over vastly expanded areas and will likely create three interrelated challenges for sustainable weed management. First, crops with stacked herbicide resistance are likely to increase the severity of resistant weeds. Second, these crops will facilitate a significant increase in herbicide use, with potential negative consequences for environmental quality. Finally, the short-term fix provided by the new traits will encourage continued neglect of public research and extension in integrated weed management. Here, we discuss the risks to sustainable agriculture from the new resistant crops and present alternatives for research and policy.

http://bioscience.oxfordjournals.org/content/62/1/75.short

Druille, M.; Cabello, M.; Omacini, M.; Golluscio, R. 2013. Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. *Applied Soil Ecology*, 64: 99–103.

Glyphosate is the most widely used herbicide in the world, but its effects on non-target organisms, such as arbuscular mycorrhizal fungi (AMF), are unclear. No studies have been found that made reference to effects of glyphosate on AMF spore viability despite its importance as a source of propagules for the perpetuation and spread of AMF in the system. The objective of this study was to evaluate the effect of glyphosate application on AMF spore viability, and their ability to colonize roots. Soil samples were collected from a grassland area located in the Flooding Pampa region (Argentina). We evaluated three herbicide rates: 0, 0.26 and 1× recommended field rate, 10 and 30 days after application. Part of the soil from each tray was used to estimate the spore viability, and the remainder was used as substrate for growing *Lolium multiflorum* Lam. One month after sowing, total root colonization and percentage of arbuscules and vesicles were determined. The spore viability in herbicide untreated soils was between 5.8- and 7.7-fold higher than in treated soils. This reduction was detected even when the lower rate was applied. Root colonization was significantly lower in plants grown in glyphosate treated soil than in untreated ones. A decrease in arbuscular colonization (but not in vesicles) was found in plants grown in soils treated with the highest herbicide rate. That would indicate that symbiosis functionality was affected, given that arbuscules are the main site for host–fungus nutrient exchange. The results indicate that soil residence time of glyphosate and/or its degradation products was enough to reduce AMF spore viability and their ability to colonize roots. This decrease in propagules viability may affect plant diversity, taking into account the different degrees of mycorrhizal dependency between plant species that may coexist in grassland communities.

http://www.sciencedirect.com/science/article/pii/S0929139312002466

Druille, M.; Omacini, M.; Golluscio, R.; Cabello, M. 2013. Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application. *Applied Soil Ecology***,** Volume 72, Pages 143–149.

Glyphosate is a systemic non-selective herbicide, the most widely used in the world. Alongside with its use in agricultural and forestry systems, this herbicide is used in grasslands in late summer with the aim of promoting winter species with the consequent increase in stocking rate. However, its effects on non-target organisms, such as arbuscular mycorrhizal fungi (AMF), are unclear. Arbuscular mycorrhizal fungi (AMF) colonize the root of more than 80% of terrestrial plants, improving their growth and survival, and therefore playing a key role in ecosystem structure and function. The aim of this work was to investigate the possible pathways through which glyphosate application affects AMF spores viability and root colonization in grassland communities. Our hypothesis is that glyphosate application can damage AMF directly (through contact with spores and external hyphae) or indirectly through the changes it generates on host plants. The experiment had a factorial array with three factors: (1) plant species, at two levels (*Paspalum dilatatum* and*Lotus tenuis*), (2) doses of glyphosate, at three levels (0 l ha^{-1}, 0.8 l ha^{-1} and 3 l ha^{-1}), and (3) application site, at two levels: soil (direct pathway) and plant foliage (indirect pathway). Spore viability was reduced even under the lowest glyphosate rate, but only when it was applied on the soil. Total root colonization for both species was similarly decreased when glyphosate was applied to plant foliage or on soil, with no difference between 0.8 and 3 l ha^{-1}. The number of arbuscules was 20% lower when glyphosate was applied on plant foliage, than when it was applied on the soil. Our findings illustrate that glyphosate application negatively affects AMF functionality in grasslands, due to different causes depending on the herbicide application site. While, under field conditions, the occurrence of direct and/or indirect pathways will depend on the plant cover at the time of glyphosate application, the consequences of this practice on the plant community structure will vary with the mycorrhizal dependence of the species composition regardless of the pathway involved.

http://www.sciencedirect.com/science/article/pii/S0929139313001868

3 Riscos de disseminação/contaminação transgênica em espécies não agrícolas[49]

O fluxo gênico representa uma das principais vias de contaminação da biodiversidade, com transgenes. A introgressão desses transgenes em populações de plantas silvestres e semisselvagens (populações ferais) pode conferir vantagem adaptativa para determinadas espécies – o que resultará em desequilíbrios ecológicos nas redes tróficas onde tais espécies se inserem – ou, ao contrário, podem conferir alguma desvantagem adaptativa, enfraquecendo essas populações em determinado ambiente. As consequências ecológicas, em qualquer caso, dependerão da repercussão do caso original sobre outras espécies, associadas à primeira.

Ao se analisar consequências ecológicas de uma transferência potencial de genes (e transgenes) – por meio da reprodução sexuada ou da transferência horizontal –, o caráter repassado (fenótipo) à espécie receptora deve ser interpretado como aspecto-chave da avaliação do risco. Nesse sentido, genes responsáveis pela síntese de toxinas inse-

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[49] Referências sobre riscos associados à disseminação de transgenes em espécies agrícolas estão disponíveis no item 4 da Parte 2.

ticidas ou de outras moléculas biocidas, pela resistência a antibióticos, ou ainda por algum ganho/redução de competitividade (maior ou menor resistência aos estresses hídricos ou salinos, por exemplo), não serão ecologicamente neutros. Portanto, causarão distúrbios em conexões de redes, entre as espécies envolvidas, que merecerão ser devidamente analisados.

Enfim, muitos cenários de risco podem vir a ocorrer quando se considera o meio ambiente como potencial reservatório-distribuidor de transgenes. Por exemplo, a possibilidade de que moléculas farmacêuticas venham a ser encontradas nas cadeias alimentares, por meio da disseminação de transgenes escapados de plantas GM biofarmacêuticas (via fecundação cruzadas com plantas silvestres e/ou ferais aparentadas, ou via Transferência Horizontal de Genes -THG - em bactérias do solo), representa riscos graves e irreversíveis, bem além dos potenciais distúrbios ecológicos acima mencionados.

3.1 Fluxo gênico por polinização[50]

Cabe lembrar que a hibridação, com ou sem introgressão de genes, entre espécies manipuladas pelo homem e espécies nativas, ameaça a segurança alimentar e a conservação da biodiversidade. Esse tema é discutido nos artigos a seguir.

Rhymer, J.; Simberloff, D. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systeatics*, 27:83–109.

Nonindigenous species can bring about a form of extinction of native flora and fauna by hybridization and introgression either through purposeful introduction by humans or through habitat modification, bringing previously isolated species into contact. These phenomena can be especially problematic for rare species coming into contact with more abundant ones. Increased use of molecular techniques focuses attention on the extent of this underappreciated problem that is not always apparent from morphological observations alone. Some degree of gene flow is a normal, evolutionarily constructive process, and all constellations of genes and genotypes cannot be preserved. However, hybridization with or without introgression may, nevertheless, threaten a rare species' existence.

http://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.27.1.83?journalCode=ecolsys.1

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[50] Na Parte 2 item 4 existem outras referências sobre fluxo gênico entre espécies aparentadas.

Allendorf, F.; Leary, R.; Spruell, P.; Wenburg, J. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution*, Vol.16 No.11.

Rates of hybridization and introgression are increasing dramatically worldwide because of translocations of organisms and habitat modifications by humans. Hybridization has contributed to the extinction of many species through direct and indirect means. However, recent studies have found that natural hybridization has played an important role in the evolution of many plant and animal taxa. Determining whether hybridization is natural or anthropogenic is crucial for conservation, but is often difficult to achieve. Controversy has surrounded the setting of appropriate conservation policies to deal with hybridization and introgression. Any policy that deals with hybrids must be flexible and must recognize that nearly every situation involving hybridization is different enough that general rules are not likely to be effective. We provide a categorization of hybridization to help guide management decisions

http://www.cell.com/trends/ecology-evolution/abstract/S0169-5347(01)02290-X?_returnURL=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS016953470102290X%3Fshowall%3Dtrue

Campbell, L.; Allison, A.; Ridley, C. 2006. Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment. *Ecology Letters*, Volume 9, Issue 11, Pages: 1198–1209.

Crop-wild hybridization may produce offspring with lower fitness than their wild parents due to deleterious crop traits and outbreeding depression. Over time, however, selection for improved fitness could lead to greater invasiveness of hybrid taxa. To examine evolutionary change in crop-wild hybrids, we established four wild (Raphanus raphanistrum) and four hybrid radish populations (R. raphanistrum x Raphanus sativus) in Michigan (MI), USA. Hybrid and wild populations had similar growth rates over four generations, and pollen fertility of hybrids improved. We then measured hybrid and wild fitness components in two common garden sites within the geographical range of wild radish [MI and California (CA)]. Advanced generation hybrids had slightly lower lifetime fecundity than wild plants in MI but exhibited c. 270% greater lifetime fecundity and c. 22% greater survival than wild plants in CA. Our results support the hypothesis that crop-wild hybridization may create genotypes with the potential to displace parental taxa in new environments.

http://www.ncbi.nlm.nih.gov/pubmed/17040322

Fitzpatrick, B.; Johnson, J.; Kump, D.; Smith, J.; Voss, S.; Shaffer, H. 2010. Rapid spread of invasive genes into a threatened native species. *PNAS*, vol. 107, no. 8, 3606–3610.

When introduced or cultivated plants or animals hybridize with their native relatives, the spread of invasive genes into native populations might have biological, aesthetic, and legal implications. Models suggest that the rate of displacement of native by invasive alleles can be rapid and inevitable if they are favored by natural selection. We document the spread of a few introduced genes 90 km into a threatened native species (the California Tiger Salamander) in 60 years. Meanwhile, a majority of genetic markers (65 of 68) show little evidence of spread beyond the region where introductions occurred. Using computer simulations, we found that such a pattern is unlikely to emerge by chance among selectively neutral markers. Therefore, our results imply that natural selection has favored both the movement and fixation of these exceptional invasive alleles. The legal status of introgressed populations (native populations that are slightly genetically modified) is unresolved by the US Endangered Species Act. Our results illustrate that genetic and ecological factors need to be carefully weighed when considering different criteria for protection, because different rules could result in dramatically different geographic areas and numbers of individuals being protected.

Artigo completo disponível em http://www.pnas.org/content/107/8/3606.full

Os prejuízos socioambientais podem ser maiores quando o fluxo gênico ocorre desde PGMs até espécies, aparentadas ou não, da agrobiodiversidade local.

Gepts, P.; Papa, R. 2003. Possible effects of (trans)gene flow from crops on the genetic diversity from landraces and wild relatives. *Environ Biosafety Res*, (2): 89–103.

Gene flow is a potential concern associated with the use of transgenic crops because it could affect genetic diversity of related landraces and wild relatives. This concern has taken on added importance with the looming introduction of transgenic crops in centers of crop domestication (Mexico, China) and those producing pharmaceutical compounds. For gene flow to take place among cultivars and their wild relatives, several steps have to be fulfilled, including the presence of cultivars or wild relatives within pollen or seed dispersal range, the ability to produce viable and fertile hybrids, at least partial overlap in flowering time, actual gene flow by pollen or seed, and the establishment of crop genes in the domesticated or wild recipient populations. In contrast with domestication genes, which often make crops less adapted to natural ecosystems, transgenes frequently represent gains of function, which might release wild relatives from constraints that limit their fitness. In most sexually reproducing organisms, the chromosomal region affected by selection of a single gene amounts to a small percentage of the total genome size. Because of gene flow, the level of genetic diversity present in the domesticated gene pool becomes a crucial factor affecting the genetic diversity of the wild gene pool. For some crops, such as cotton and maize, the introduction of transgenic technologies has led to a consolidation of the seed industry and a reduction in the diversity of the elite crop gene pool. Thus, diversity in improved varieties grown by farmers needs to be monitored. Several areas deserve further study, such as the actual magnitude of gene flow and its determinants in different agroecosystems, the long-term effects of gene flow on genetic diversity both across gene pools and within genomes, the expression of transgenes in new genetic backgrounds, and the effects of socio-economic factors on genetic diversity.

http://www.ncbi.nlm.nih.gov/pubmed/15612275

3.2 Desequilíbrios ecológicos com disseminação de transgenes em espécies silvestres[51]

Uma vez que uma planta transgênica está cultivada em campo, em escala comercial ou experimental, não haverá forma ou possibilidade de impedir o escape de transgenes para o meio ambiente. No item 3.3 da Parte 2 foram apresentados alguns casos de transferência bem-sucedida de transgenes para populações de plantas ruderais consideradas daninhas.

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[51] Cabe ressaltar que os riscos associados ao potencial de invasão de espécies que sofreram de introgressão de transgenes são maiores (e mais facilmente identificados) em populações animais. O exemplo do salmão transgênico com maior taxa de crescimento não deixa dúvida sobre o caractere negativo dos impactos ecológicos de um potencial escape de tais indivíduos/transgenes sobre populações nativas de peixes e outros organismos aquáticos.

Os artigos a seguir mostram que pode haver introgressão de transgenes em espécies silvestres geneticamente aparentadas (que não sofreram processo de domesticação), gerando riscos de desequilíbrios ecológicos de diferentes tipos em ambientes naturais e seminaturais. O potencial de invasão dessas "novas espécies" deve vir a ser uma das maiores preocupações da análise do risco relativamente a esse assunto.

REV - Ellstrand, N.; Schierenbeck, K. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *PNAS*, vol. 97, no. 13, 7043–7050.

Invasive species are of great interest to evolutionary biologists and ecologists because they represent historical examples of dramatic evolutionary and ecological change. Likewise, they are increasingly important economically and environmentally as pests. Obtaining generalizations about the tiny fraction of immigrant taxa that become successful invaders has been frustrated by two enigmatic phenomena. Many of those species that become successful only do so (*i*) after an unusually long lag time after initial arrival, and/or (*ii*) after multiple introductions. We propose an evolutionary mechanism that may account for these observations. Hybridization between species or between disparate source populations may serve as a stimulus for the evolution of invasiveness. We present and review a remarkable number of cases in which hybridization preceded the emergence of successful invasive populations. Progeny with a history of hybridization may enjoy one or more potential genetic benefits relative to their progenitors. The observed lag times and multiple introductions that seem a prerequisite for certain species to evolve invasiveness may be a correlate of the time necessary for previously isolated populations to come into contact and for hybridization to occur. Our examples demonstrate that invasiveness can evolve. Our model does not represent the *only* evolutionary pathway to invasiveness, but is clearly an underappreciated mechanism worthy of more consideration in explaining the evolution of invasiveness in plants.

Artigo completo disponível em http://www.pnas.org/content/97/13/7043.full

Warwick, S.; Simard, M.; Légère, A.; Beckie, H.; Braun, L.; Zhu, B.; Mason, P.; Séguin-Swartz, G.; Stewart, C. 2003. Hybridization between transgenic Brassica napus L. and its wild relatives: *Brassica rapa L.*, *Raphanus raphanistrum L.*, *Sinapis arvensis L.*, and *Erucastrum gallicum* (Willd.) O.E. Schulz. *Theor Appl Genet*, 107(3):528-39.

The frequency of gene flow from Brassica napus L. (canola) to four wild relatives, Brassica rapa L., Raphanus raphanistrum L., Sinapis arvensis L. and Erucastrum gallicum (Willd.) O.E. Schulz, was assessed in greenhouse and/or field experiments, and actual rates measured in commercial fields in Canada. Various marker systems were used to detect hybrid individuals: herbicide resistance traits (HR), green fluorescent protein marker (GFP), species-specific amplified fragment length polymorphisms (AFLPs) and ploidy level. Hybridization between B. rapa and B. napus occurred in two field experiments (frequency approximately 7%) and in wild populations in commercial fields (approximately 13.6%). The higher frequency in commercial fields was most likely due to greater distance between B. rapa plants. All F(1) hybrids were morphologically similar to B. rapa, had B. napus- and B. rapa-specific AFLP markers and were triploid (AAC, 2n=29 chromosomes). They had reduced pollen viability (about 55%) and segregated for both self-incompatible and self-compatible individuals (the latter being a B. napus trait). In contrast, gene flow between R. raphanistrum and B. napus was very rare. A single R. raphanistrum x B. napus F1 hybrid was detected in 32,821 seedlings from the HR B. napus field experiment. The hybrid was morphologically similar to R. raphanistrum except for the presence of valves, a B. napus trait, in the distorted seed pods. It had a genomic structure consistent with the fusion of an unreduced gamete of R. raphanistrum and a reduced gamete of B. napus (RrRrAC, 2n=37), both B. napus- and R. raphanistrum-specific AFLP markers, and had <1% pollen viability. No hybrids were detected in the greenhouse experiments (1,534 seedlings), the GFP field experiment (4,059 seedlings) or in commercial fields in Québec and Alberta (22,114 seedlings). No S. arvensis or E. gallicum x B. napus hybrids were detected (42,828 and 21,841 seedlings, respectively) from commercial fields in Saskatchewan. These findings suggest that the probability of gene flow from transgenic B. napus to R. raphanistrum, S. arvensis or E. gallicum is very low (<2-5 x 10(-5)). However, transgenes can disperse in the environment via wild B. rapa in eastern Canada and possibly via commercial B. rapa volunteers in western Canada.

http://www.ncbi.nlm.nih.gov/pubmed/12721639

REV - Ellstrand, N. 2003. Current knowledge of gene flow in plants: implications for transgene flow. *Phil Trans R Soc Lond*, B, 358, 1163-1170.

Plant evolutionary biologists' view of gene flow and hybridization has undergone a revolution. Twenty-five years ago, both were considered rare and largely inconsequential. Now gene flow and hybridization are known to be idiosyncratic, varying with the specific populations involved. Gene flow typically occurs at evolutionarily significant rates and at significant distances. Spontaneous hybridization occasionally has important applied consequences, such as stimulating the evolution of more aggressive invasives and increasing the extinction risk for rare species. The same problems have occurred for spontaneous hybridization between crops and their wild relatives. These new data have implications for transgenic crops: (i) for most crops, gene flow can act to introduce engineered genes into wild populations; (ii) depending on the specific engineered gene(s) and populations involved, gene flow may have the same negative impacts as those observed for traditionally improved crops; (iii) gene flow's idiosyncratic nature may frustrate management and monitoring attempts; and (iv) intercrop transgene flow, although rarely discussed, is equally worthy of study.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1693201/pdf/12831483.pdf

Vacher, C.; Weis, A.; Hermann, D.; Kossler, T.; Young, C.; Hochbert, M. 2004. Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (*Brassica rapa*). *Theor Appl Genet*, 109 (4): 806-14.

The inevitable escape of transgenic pollen from cultivated fields will lead to the emergence of transgenic crop-wild plant hybrids in natural patches of wild plants. The fate of these hybrids and that of the transgene depend on their ability to compete with their wild relatives. Here we study ecological factors that may enhance the fitness of genetically modified hybrids relative to wild plants for a Bacillus thuringiensis (Bt) transgene conferring resistance to insects. Mixed stands of wild plants and first-generation hybrids were grown under different conditions of herbivore pressure and density, with Bt oilseed rape (Brassica napus) as the crop and B. rapa as the wild recipient. Biomass and fitness components were measured from plant germination to the germination of their offspring. The frequency of transgenic seedlings in the offspring generation was estimated using the green fluorescent protein marker. The biomass of F(1) Bt-transgenic hybrids relative to that of wild-type plants was found to be sensitive to both plant density and herbivore pressure, but herbivore pressure appeared as the major factor enhancing their relative fitnesses. In the absence of herbivore pressure, Bt hybrids produced 6.2-fold fewer seeds than their wild neighbors, and Bt plant frequency fell from 50% to 16% within a single generation. Under high herbivore pressure, Bt hybrids produced 1.4-fold more seeds, and Bt plant frequency was 42% in the offspring generation. We conclude that high-density patches of highly damaged wild plants are the most vulnerable to Bt-transgene invasion. They should be monitored early to detect potential transgene spread.

http://www.ncbi.nlm.nih.gov/pubmed/15340690

REV - Chapman, M.; Burke, J. 2006. Letting the gene out of the bottle: the population genetics of genetically modified crops. *New Phytologist*, 170, 429–443.

Genetically modified (GM) plants are rapidly becoming a common feature of modern agriculture. This transition to engineered crops has been driven by a variety of potential benefits, both economic and ecological. The increase in the use of GM crops has, however, been accompanied by growing concerns regarding their potential impact on the environment. Here, we focus on the escape of transgenes from cultivation via crop x wild hybridization. We begin by reviewing the literature on natural hybridization, with particular reference to gene flow between crop plants and their wild relatives. We further show that natural selection, and not the overall rate of gene flow, is the most important factor governing the spread of favorable alleles. Hence, much of this review focuses on the likely effects of transgenes once they escape. Finally, we consider strategies for transgene containment.

http://www.ncbi.nlm.nih.gov/pubmed/16626466

3.3. Fluxo gênico por transferência horizontal de genes (THG)

Outra possibilidade de escape de material transgênico para o meio ambiente, talvez mais grave porque se revela independentemente do ser humano e das suas atividades, diz respeito à Transferência Horizontal de (trans)Genes por meio de vírus, transposons e plasmídeos. Como ressaltado no item 3.1 da Parte 1[52] deste documento, a ocorrência de THG é fenômeno que ocorre de modo permanente na escala evolutiva, constituindo maior fonte de impulso à diversidade genética do que o próprio processo de mutação e sendo responsável por alterações na composição genética da maioria das espécies presentes na biosfera.

Existem diversas publicações sobre esse tema. No caso da avaliação de risco em plantas transgênicas, as abordagens parecem se concentrar sobre a possibilidade de introgressão do transgene (ou outras sequências genômicas do cassete de expressão, tais como os genes de resistência a antibióticos) à base genética de outros organismos, aparentados ou geneticamente distantes do primeiro. Esses autores ressaltam a dificuldade de observar tal fenômeno em experimentos, bem como a complexidade associada ao dimensionamento e ao mapeamento dos riscos associados, em que pese sua frequente ocorrência.

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[52] Vale conferir também o item 3.3 da Parte 1 referente aos riscos específicos de transferência horizontal de genes associados ao uso de *Agrobacterium tumefaciens* para a transformação de plantas.

Gebhard, F.; Smalla, K. 1998. Transformation of *acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology*, p. 1550–1554.

The ability of *Acinetobacter* sp. strain BD413(pFG4Δ*npt*II) to take up and integrate transgenic plant DNA based on homologous recombination was studied under optimized laboratory conditions. Restoration of *npt*II, resulting in kanamycin-resistant transformants, was observed with plasmid DNA, plant DNA, and homogenates carrying the gene *npt*II. Molecular analysis showed that some transformants not only restored the 317-bp deletion but also obtained additional DNA.

Artigo completo disponível em http://aem.asm.org/content/64/4/1550.full

Nielsen, K.; Bones, A.; Smalla, K.; van Elsas, J. 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria a-rare event? *FEMS Microbiology Reviews*, 22: 79-103.

Today, 12 years after the first field release of a genetically modified plant (GMP), over 15,000 field trials at different locations have been performed. As new and unique characteristics are frequently introduced into GMPs, risk assessment has to be performed to assess their ecological impact. The possibilities of horizontal gene transfer (HGT; no parent-to-offspring transfer of genes) from plants to microorganisms are frequently evaluated in such risk assessments of GMPs before release into the field. In this review we indicate why putative HGT from plants to terrestrial (soil and plant associated) bacteria has raised concern in biosafety evaluations. Further, we discuss possible pathways of HGT from plants to bacteria, outline the barriers to HGT in bacteria, describe the strategies used to investigate HGT from plants to bacteria and summarize the results obtained. Only a few cases of HGT from eukaryotes such as plants to bacteria have been reported to date. These cases have been ascertained after comparison of DNA sequences between plants and bacteria. Although experimental approaches in both field and laboratory studies have not been able to confirm the occurrence of such HGT to naturally occurring bacteria, recently two studies have shown transfer of marker genes from plants to bacteria based on homologous recombination. The few examples of HGT indicated by DNA sequence comparisons suggest that the frequencies of evolutionarily successful HGT from plants to bacteria may be extremely low. However, this inference is based on a small number of experimental studies and indications found in the literature. Transfer frequencies should not be confounded with the likelihood of environmental implications, since the frequency of HGT is probably only marginally important compared with the selective force acting on the outcome. Attention should therefore be focused on enhancing the understanding of selection processes in natural environments. Only an accurate understanding of these selective events will allow the prediction of possible consequences of novel genes following their introduction into open environments.

http://www.ncbi.nlm.nih.gov/pubmed/9729765

Gebhard, F.; Smalla, K. 1999. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology*, 28 (3): 261-72.

Field releases of transgenic rizomania-resistant sugar beet (*Beta vulgaris*) plants were accompanied by a study of the persistence of DNA from transgenic sugar beet litter in soil and of horizontal gene transfer of plant DNA to bacteria. The transgenic sugar beets contained the marker genes *npt*II and *bar* under the control of the bidirectional TR1/2 promoter conferring kanamycin (Km) and glufosinate ammonium resistance to the plant. Primer systems targeting the construct allowed the specific and sensitive detection of the transgenic DNA in soil. Soil samples were analyzed by cultivation of bacteria on nonselective and Km-selective media to determine the proportion of Km-resistant bacteria and to monitor the culturable fraction for incorporation of transgenic plant DNA. To detect the presence of transgenic DNA independently from cultivation, total soil DNA was extracted and amplified by PCR with three different primer sets specific for the transgenic DNA. Long-term persistence of transgenic DNA could be shown under field conditions (up to 2 years) and also in soil microcosms with introduced transgenic plant DNA. No construct-specific sequences were detected by dot blot hybridizations of bacterial isolates. The experimental limitations of detecting horizontal gene transfer from plants to bacteria under field conditions are discussed.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6941.1999.tb00581.x/pdf

Nielsen, K.; van Elsas, J.; Smalla, K. 2000. Transformation of *Acinetobacter* sp. Strain BD413(pFG4Δ*nptII*) with Transgenic Plant DNA in Soil Microcosms and Effects of Kanamycin on Selection of Transformants. *Applied and Environmental Microbiology*; 66(3): 1237–1242.

Here we show that horizontal transfer of DNA, extracted from transgenic sugar beets, to bacteria, based on homologous recombination, can occur in soil. Restoration of a 317-bp-deleted *nptII* gene in *Acinetobacter* sp. strain BD413(pFG4) cells incubated in sterile soil microcosms was detected after addition of nutrients and transgenic plant DNA encoding a functional *nptII* gene conferring bacterial kanamycin resistance. Selective effects of the addition of kanamycin on the population dynamics of *Acinetobacter* sp. cells in soil were found, and high concentrations of kanamycin reduced the CFU of *Acinetobacter* sp. cells from 10^9 CFU/g of soil to below detection. In contrast to a chromosomal *nptII*-encoded kanamycin resistance, the pFG4-generated resistance was found to be unstable over a 31-day incubation period in vitro.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC91972/

REV - Heinemann, J.; Travick, T. 2004. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. *Nature Biotechnology*, volume 22 number 9.

Transgenic crops are approved for release in some countries, while many more countries are wrestling with the issue of how to conduct risk assessments. Controls on field trials often include monitoring of horizontal gene transfer (HGT) from crops to surrounding soil microorganisms. Our analysis of antibiotic-resistant bacteria and of the sensitivity of current techniques for monitoring HGT from transgenic plants to soil microorganisms has two major implications for field trial assessments of transgenic crops: first, HGT from transgenic plants to microbes could still have an environmental impact at a frequency approximately a trillion times lower than the current risk assessment literature estimates the frequency to be; and second, current methods of environmental sampling to capture genes or traits in a recombinant are too insensitive for monitoring evolution by HGT. A model for HGT involving iterative short-patch events explains how HGT can occur at high frequencies but be detected at extremely low frequencies.

Artigo completo disponível em http://www.keine-gentechnik.de/bibliothek/naturschutz/studien/nature_biotechnology_monitoring_horizontal_gene_transfer_2_040901.pdf

MOD - Nielsen, K.; Townsend, J. 2004. Monitoring and modeling horizontal gene transfer. *Nature Biotechnology*, Volume 22 number 9.

Monitoring efforts have failed to identify horizontal gene transfer (HGT) events occurring from transgenic plants into bacterial communities in soil or intestinal environments. The lack of such observations is frequently cited in biosafety literature and by regulatory risk assessment. Our analysis of the sensitivity of current monitoring efforts shows that studies to date have examined potential HGT events occurring in less than 2 g of sample material, when combined. Moreover, a population genetic model predicts that rare bacterial transformants acquiring transgenes require years of growth to out-compete wild-type bacteria. Time of sampling is there-fore crucial to the useful implementation of monitoring. A population genetic approach is advocated for elucidating the necessary sample sizes and times of sampling for monitoring HGT into large bacterial populations. Major changes in current monitoring approaches are needed, including explicit consideration of the population size of exposed bacteria, the bacterial generation time, the strength of selection acting on the transgene-carrying bacteria, and the sample size necessary to verify or falsify the HGT hypotheses tested.

http://www.ncbi.nlm.nih.gov/pubmed/15340481

REV - Pontiroli, A.; Simonet, P.; Frostegard, A.; Vogel, T.; Monier, J. 2007. Fate of transgenic plant DNA in the environment. *Environ Biosafety Res*, 6(1-2): 15-35.

This review addresses the possible ecological effects of transgenic plants on micro-organisms in the field, hence, in the phytosphere and in the soil matrix. The important steps involved in the interaction between plant DNA and bacteria and the factors that influence the horizontal gene transfer (HGT) process will be discussed. HGT is a process in which two partners are involved, even if indirectly. In the first section, aspects concerning bacteria, such as their physico-chemical, biological and genetic characteristics, are described. Parameters affecting transgenic DNA fate in the environment are described in the second section. Subsequently, terrestrial habitats are evaluated in terms of their capacity to favor horizontal gene transfer. Finally, we focused on several studies in order to evaluate possible perturbations of soil bacterial community composition due to cultivation of transgenic plants in the field.

Artigo completo disponível em http://www.ebr-journal.org/articles/ebr/pdf/2007/01/ebr0626.pdf

Ulker, B.; Li, Y.; Rosso, M.; Logemann, E.; Somssich, I.; Weisshaar, B. 2008. T-DNA–mediated transfer of *Agrobacterium tumefaciens* chromosomal DNA into plants. *Nature Biotechnology*, volume 26, number 9.

Besides the well-documented integration of DNA flanked by the transfer DNA borders, occasional insertion of fragments from the tumor-inducing plasmid into plant genomes has also been reported during Agrobacterium tumefaciens-mediated transformation. We demonstrate that large (up to approximately 18 kb) gene-bearing fragments of Agrobacterium chromosomal DNA (AchrDNA) can be integrated into Arabidopsis thaliana genomic DNA during transformation. One in every 250 transgenic plants may carry AchrDNA fragments. This has implications for horizontal gene transfer and indicates a need for greater scrutiny of transgenic plants for undesired bacterial DNA.

http://www.ncbi.nlm.nih.gov/pubmed/18758448

REV - Brigulla, M.; Wackernagel, W. 2010. Molecular aspects of gene transfer and foreign DNA acquisition in prokaryotes with regard to safety issues. *Applied microbiology and biotechnology*, 86(4): 1027-1041.

Horizontal gene transfer (HGT) is part of prokaryotic life style and a major factor in evolution. In principle, any combinations of genetic information can be explored via HGT for effects on prokaryotic fitness. HGT mechanisms including transformation, conjugation, transduction, and variations of these plus the role of mobile genetic elements are summarized with emphasis on their potential to translocate foreign DNA. Complementarily, we discuss how foreign DNA can be integrated in recipient cells through homologous recombination (HR), illegitimate recombination (IR), and combinations of both, site-specific recombination, and the reconstitution of plasmids. Integration of foreign DNA by IR is very low, and combinations of IR with HR provide intermediate levels compared to the high frequency of homologous integration. A survey of studies on potential HGT from various transgenic plants indicates very rare transfer of foreign DNA. At the same time, in prokaryotic habitats, genes introduced into transgenic plants are abundant, and natural HGT frequencies are relatively high providing a greater chance for direct transfer instead of via transgenic plants. It is concluded that potential HGT from transgenic plants to prokaryotes is not expected to influence prokaryotic evolution and to have negative effects on human or animal health and the environment.

http://www.ncbi.nlm.nih.gov/pubmed/20191269

REV - Nielsen, K.; Bøhn, T.; Townsend, J. 2014. Detecting rare gene transfer events in bacterial populations. *Frontiers in Microbiology*, Volume 4, Article 415.

Horizontal gene transfer (HGT) enables bacteria to access, share, and recombine genetic variation, resulting in genetic diversity that cannot be obtained through mutational processes alone. In most cases, the observation of evolutionary successful HGT events relies on the outcome of initially rare events that lead to novel functions in the new host, and that exhibit a positive effect on host fitness. Conversely, the large majority of HGT events occurring in bacterial populations will go undetected due to lack of replication success of transformants. Moreover, other HGT events that would be highly beneficial to new hosts can fail to ensue due to lack of physical proximity to the donor organism, lack of a suitable gene transfer mechanism, genetic compatibility, and stochasticity in tempo-spatial occurrence. Experimental attempts to detect HGT events in bacterial populations have typically focused on the transformed cells or their immediate offspring. However, rare HGT events occurring in large and structured populations are unlikely to reach relative population sizes that will allow their immediate identification; the exception being the unusually strong positive selection conferred by antibiotics. Most HGT events are not expected to alter the likelihood of host survival to such an extreme extent, and will confer only minor changes in host fitness. Due to the large population sizes of bacteria and the time scales involved, the process and outcome of HGT are often not amenable to experimental investigation. Population genetic modeling of the growth dynamics of bacteria with differing HGT rates and resulting fitness changes is therefore necessary to guide sampling design and predict realistic time frames for detection of HGT, as it occurs in laboratory or natural settings. Here we review the key population genetic parameters, consider their complexity and highlight knowledge gaps for further research.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3882822/

A presença de DNA recombinante no meio ambiente, após decomposição de material biológico transgênico, representa um dos pontos de partida da transferência horizontal de genes. Os artigos listados a seguir acusam presença de transgenes/DNA recombinante em diversos ecossistemas e agrossistemas onde se cultivam plantas transgênicas, bem como em cadeias tróficas de micro-organismos e artrópodes dos arredores, ou ainda em cursos d'água. Os estudos sugerem que tal disseminação pode ser irrefreável.

Lerat, S.; Gulden, R.; Hart, M.; Powell, J.; England, L.; Pauls, K.; Swanton, C.; Klironomos, J.; Trevors, J. 2007. Quantification and persistence of recombinant DNA of Roundup Ready corn and soybean in rotation. *Journal of Agricultural and Food Chemistry*, 55(25): 10226-10231.

The presence of the recombinant *cp4 epsps* gene from Roundup Ready (RR) corn and RR soybean was quantified using real-time PCR in soil samples from a field experiment growing RR and conventional corn and soybean in rotation. RR corn and RR soybean *cp4 epsps* persisted in soil for up to 1 year after seeding. The concentration of recombinant DNA in soil peaked in July and August in RR corn and RR soybean plots, respectively. A small fraction of soil samples from plots seeded with conventional crops contained recombinant DNA, suggesting transgene dispersal by means of natural process or agricultural practices. This research will aid in the understanding of the persistence of recombinant DNA in agricultural cropping systems.

http://pubs.acs.org/doi/abs/10.1021/jf072457z?journalCode=jafcau

REV - Nielsen, K.; Johnsen, P; Bensasson, D.; Daffonchio, D. 2007. Release and persistence of extracellular DNA in the environment. *Environmental Biosafety Research*, 6: 37–53.

The introduction of genetically modified organisms (GMOs) has called for an improved understanding of the fate of DNA in various environments, because extracellular DNA may also be important for transferring genetic information between individuals and species. Accumulating nucleotide sequence data suggest that acquisition of foreign DNA by horizontal gene transfer (HGT) is of considerable importance in bacterial evolution. The uptake of extracellular DNA by natural transformation is one of several ways bacteria can acquire new genetic information given sufficient size, concentration and integrity of the DNA. We review studies on the release, breakdown and persistence of bacterial and plant DNA in soil, sediment and water, with a focus on the accessibility of the extracellular nucleic acids as substrate for competent bacteria. DNA fragments often persist over time in many environments, thereby facilitating their detection and characterization. Nevertheless, the long-term physical persistence of DNA fragments of limited size observed by PCR and Southern hybridization often contrasts with the short-term availability of extracellular DNA to competent bacteria studied in microcosms. The main factors leading to breakdown of extracellular DNA are presented. There is a need for improved methods for accurately determining the degradation routes and the persistence, integrity and potential for horizontal transfer of DNA released from various organisms throughout their lifecycles.

Artigo completo disponível em http://www.ebr-journal.org/articles/ebr/pdf/2007/01/ebr0608.pdf

Hart, M.; Powell, J.; Gulden, R.; Levy-Booth, D.; Dunfield, K.; Pauls, K.; Swanton, C; Klironomos, J.; Trevors, J. 2009. Detection of transgenic *cp4 epsps* genes in the soil food web. *Agronomy for Sustainable Development*, 29, 497–501.

The persistence and movement of transgenic DNA in agricultural and natural systems is largely unknown. This movement poses a threat of horizontal gene transfer and possible proliferation of genetically modified DNA into the general environment. To assess the persistence of transgenic DNA in a field of Roundup Ready® corn, we quantified the presence of the transgene for glyphosate tolerance within a soil food web. Using quantitative real-time PCR, we identified the *cp4 epsps*transgene in bulk soil microarthropods, nematodes, macroarthropods and earthworms sampled within the corn cropping system. We found evidence of the transgene at all dates and in all animal groups. Transgenic DNA concentration in animal was significantly higher than that of background soil, suggesting the animals were feeding directly on transgenic plant material. It remains to be tested whether this DNA was still within the plant residues, present as free, extracellular DNA or had already undergone genetic transformation into competent bacterial cells. These results are the first to demonstrate the persistence of transgenic crop DNA residues within a food web.

Artigo completo disponível em http://soilmicrobialecology.ok.ubc.ca/Group_Site/Faculty/Entries/2010/6/29_Miranda_Hart,_Ph.D._files/Hart%20et%20al.%202009%20Agron..pdf

Chen, J.; Jin, M.; Qiu Z.; Guo, C.; Chen, Z.; Shen, Z.; Wang, X.; Li, J. 2012. A survey of drug resistance bla genes originating from synthetic plasmid vectors in six Chinese rivers. *Environ Sci Technol*, 46(24): 13448-54. doi: 10.1021/es302760s.

Antibiotic resistance poses a significant challenge to human health and its rate continues to rise globally. While antibiotic-selectable synthetic plasmid vectors have proved invaluable tools of genetic engineering, this class of artificial recombinant DNA sequences with high expression of antibiotic resistance genes presents an unknown risk beyond the laboratory setting. Contamination of environmental microbes with synthetic plasmid vector-sourced antibiotic resistance genes may represent a yet unrecognized source of antibiotic resistance. In this study, PCR and real-time quantitative PCR were used to investigate the synthetic plasmid vector-originated ampicillin resistance gene, β-lactam antibiotic (blá), in microbes from six Chinese rivers with significant human interactions. Various levels of blá were detected in all six rivers, with the highest levels in the Pearl and Haihe rivers. To validate the blá pollution, environmental plasmids in the river samples were captured by the E. coli transformants from the community plasmid metagenome. The resultant plasmid library of 205 ampicillin-resistant E. coli (transformants) showed a blá-positive rate of 27.3% by PCR. Sequencing results confirmed the synthetic plasmid vector sources. In addition, results of the Kirby-Bauer disc-diffusion test reinforced the ampicillin-resistant functions of the environmental plasmids. The resistance spectrum of transformants from the Pearl and Haihe rivers, in particular, had expanded to the third- and fourth-generation of cephalosporin drugs, while that of other transformants mainly involved first- and second-generation cephalosporins. This study not only reveals environmental contamination of synthetic plasmid vector-sourced blá drug resistance genes in Chinese rivers, but also suggests that synthetic plasmid vectors may represent a source of antibiotic resistance in humans.

http://www.ncbi.nlm.nih.gov/pubmed/23215020

Na avaliação de impactos ambientais decorrentes da transferência horizontal de (trans)genes, é importante entender seu caráter irreversível. Pesquisas recentes apontam a possibilidade de que moléculas de DNA, inclusive aquelas presentes na forma de DNA livre no meio ambiente, mantenham suas estruturas físicas durante milhares de anos e ainda assim possam vir a ser adquiridas por bactérias competentes, via THG.

Willerslev, E.; Hansen, A.; Binladen, J.; Brand, T.; Gilbert, M.; Shapiro, B.; Bunce, M.; Carsten, W.; Gilichinsky, D.; Cooper, A. 2003. Diverse plant and animal genetic records from holocene and pleistocene sediments. *Science*, vol 300.

Genetic analyses of permafrost and temperate sediments reveal that plant and animal DNA may be preserved for long periods, even in the absence of obvious macrofossils. In Siberia, five permafrost cores ranging from 400,000 to 10,000 years old contained at least 19 different plant taxa, including the oldest authenticated ancient DNA sequences known, and megafaunal sequences including mammoth, bison, and horse. The genetic data record a number of dramatic changes in the taxonomic diversity and composition of Beringian vegetation and fauna. Temperate cave sediments in New Zealand also yielded DNA sequences of extinct biota, including two species of ratite moa, and 29 plant taxa characteristic of the prehuman environment. Therefore, many sedimentary deposits may contain unique, and widespread, genetic records of paleoenvironments.

http://www.ncbi.nlm.nih.gov/pubmed/12702808

Overballe-Petersen, S.; Harms, K.; Orlando, L.; Mayar, J.; Rasmussen, S.; Dahl, T.; Rosing, M.; Poole, A.; Sicheritz-Ponten, T.; Brunak, S.; Inselmann, S.; de Vries, J.; Wackernagel, W.; Pybus, O.; Nielsen, R.; Johnsen, P.; Nielsen, K.; Willerslev, E. 2013. Bacterial natural transformation by highly fragmented and damaged DNA. *PNAS*, vol. 110, no. 4919860–19865.

DNA molecules are continuously released through decomposition of organic matter and are ubiquitous in most environments. Such DNA becomes fragmented and damaged (often <100 bp) and may persist in the environment for more than half a million years. Fragmented DNA is recognized as nutrient source for microbes, but not as potential substrate for bacterial evolution. Here, we show that fragmented DNA molecules (≥20 bp) that additionally may contain abasic sites, cross-links, or miscoding lesions are acquired by the environmental bacterium Acinetobacter baylyi through natural transformation. With uptake of DNA from a 43,000-y-old woolly mammoth bone, we further demonstrate that such natural transformation events include ancient DNA molecules. We find that the DNA recombination is RecA recombinase independent and is directly linked to DNA replication. We show that the adjacent nucleotide variations generated by uptake of short DNA fragments escape mismatch repair. Moreover, doublenucleotide polymorphisms appear more common among genomes of transformable than nontransformable bacteria. Our findings reveal that short and damaged, including truly ancient, DNA molecules, which are present in large quantities in the environment, can be acquired by bacteria through natural transformation. Our findings open for the possibility that natural genetic exchange can occur with DNA up to several hundreds of thousands years old.

Artigo completo disponível em http://www.pnas.org/content/110/49/19860.full

Parte 4

Riscos para a saúde associados ao cultivo e/ou uso de plantas transgênicas

Após 20 anos de polêmica em relação aos riscos para a saúde associados ao consumo de plantas transgênicas, ainda não há consenso na comunidade científica sobre possibilidades de danos e limites aceitáveis de consumo, para alimentos compostos por plantas geneticamente modificadas e suas partes. Como agravante, a maior parte dos estudos que apontam segurança nutricional e toxicológica parece ter sido realizada em perspectiva de curto prazo, desprezando efeitos crônicos, sem levar em conta os pacotes tecnológicos associados (por exemplo, avaliando o consumo de milho tolerante ao glifosato com base em grãos colhidos de lavoura onde não foi utilizado o herbicida) ou mesmo sem levar em conta as proteínas transgênicas presentes na PGM Bt (por exemplo, avaliando riscos de alergenicidade com proteína Cry homóloga, extraída da bactéria, para atestar segurança de toxina similar àquela presente no milho). Estudos independentes que permitem questionar aqueles métodos estabelecem quadro preocupante, que justifica a necessidade de análises mais acuradas, de longo prazo, apoiadas por metodologias robustas e consistentes.

Quando se olha para o conjunto da literatura científica publicada em revistas especializadas, percebe-se a importância dos chamados estudos independentes e a alarmante frequência com que estes apontam para riscos significativos, ameaçadores da saúde humana e animal. Os problemas parecem claramente relacionados a impactos de subdosagens, provocando alterações crônicas que tendem a se manifestar no longo prazo. Sugere-se, dessa forma, a necessidade de coleta e disponibilidade de dados de biossegurança, bem como a facilitação de acesso a informações resultantes de estudos já concluídos e em andamento. Preocupa o fato de que entidades reguladoras orientem suas decisões a partir de publicações geradas pelas empresas, em que as informações de fundo tendem a ser ocultadas ao escrutínio da sociedade – em muitos casos os dados não são disponibilizados sequer para membros das agências reguladoras que os avaliam.

Nos artigos listados abaixo, ao revisar o conjunto de estudos toxicológicos publicados e referentes aos riscos para a saúde das plantas transgênicas, alguns autores consideram que os dados científicos disponíveis não apoiam a afirmação de consumo seguro daquelas plantas. Já, outros autores são mais taxativos em afirmar que alterações fisiológicas observadas independentemente em vários estudos não podem ser apenas fruto do acaso (em especial no que diz respeito a variáveis biológicas indicadoras de toxicidade hepático-renal), concluindo que esses produtos não são biosseguros para consumo humano e animal no longo prazo.

COM - Domingo, J. 2000. Health risks of GM foods: many opinions but few data. *Science*, 288:1748–9.

Sem resumo.
Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/Domingo2000.pdf

REV - Pryme, I.; Lembcke, R. 2003. In vivo studies on possible health consequences of genetically modified food and feed with particular regard to ingredients consisting of genetically modified plant materials. *Nutr Health*, 17: 1-8.

This synopsis reviews published in vivo studies on possible health consequences of genetically modified food and feed where the ingredients in question have consisted of genetically modified plant materials. The following, however, have not been taken into consideration:--ingredients consisting of genetically modified microorganisms or parts of animals/fish--ingredients produced by/from genetically modified organisms but without any DNA present--studies on consequences for the environment or biodiversity--in vitro studies or computer simulations. According to a Norwegian report "Gen-mat" (NOU 2000:29), and a more recent search in Medline and Citations Index, to our knowledge a total of ten studies have been published on the health effects of GM-foods and feeds. In this minireview the data made available in these published studies is discussed.

http://www.ncbi.nlm.nih.gov/pubmed/12803276

REV - Nodari, R.; Guerra, M. 2003. Transgenic plants and their products: effects, risks and food safety (Biosafety of transgenic plants). *Rev Nutr*, Campinas, 16(1):105-116.

Este trabalho aborda tópicos relacionados com plantas transgênicas, também chamadas de Organismos Geneticamente Modificados, alimentos derivados delas e segurança alimentar. As biotecnologias modernas são ferramentas de grande potencial de reprogramação dos seres vivos. Contudo, o maior problema na análise de risco destes organismos gerados pela biotecnologia é que seus efeitos não podem ser previstos em sua totalidade. Os riscos à saúde humana incluem aqueles inesperados, alergias, toxicidade e intolerância. No ambiente, as conseqüências são a transferência lateral de genes, a poluição genética e os efeitos prejudiciais a organismos não-alvo.

O princípio da equivalência substancial, até agora utilizado, deveria ser abandonado em favor de um cientificamente embasado. Com a aprovação em janeiro de 2002 do Protocolo Internacional de Biossegurança, o princípio da precaução foi estabelecido como básico e a rotulagem tornou-se obrigatória. A percepção pública obriga empresas e cientistas a um maior uso da ciência na análise de risco antes do consumo destes alimentos.

Artigo completo disponível em http://www.scielo.br/pdf/rn/v16n1/a10v16n1.pdf

REV - Domingo, J. 2007. Toxicity studies of genetically modified plants: a review of the published literature. *Crit Rev Food Sci Nutr*, 47:721–33.

According to the information reported by the WHO, the genetically modified (GM) products that are currently on the international market have all passed risk assessments conducted by national authorities. These assessments have not indicated any risk to human health. In spite of this clear statement, it is quite amazing to note that the review articles published in international scientific journals during the current decade did not find, or the number was particularly small, references concerning human and animal toxicological/health risks studies on GM foods. In this paper, the scientific information concerning the potential toxicity of GM/transgenic plants using the Medline database is reviewed. Studies about the safety of the potential use of potatoes, corn, soybeans, rice, cucumber, tomatoes, sweet pepper, peas, and canola plants for food and feed were included. The number of references was surprisingly limited. Moreover, most published studies were not performed by the biotechnology companies that produce these products. This review can be concluded raising the following question: where is the scientific evidence showing that GM plants/food are toxicologically safe?

http://www.ncbi.nlm.nih.gov/pubmed/17987446

REV - Dona, A.; Arvanitoyannis, I. 2009. Health risks of genetically modified foods. *Crit Rev Food Sci Nutr*, 49 : 164–75.

As genetically modified (GM) foods are starting to intrude in our diet concerns have been expressed regarding GM food safety. These concerns as well as the limitations of the procedures followed in the evaluation of their safety are presented. Animal toxicity studies with certain GM foods have shown that they may toxically affect several organs and systems. The review of these studies should not be conducted separately for each GM food, but according to the effects exerted on certain organs it may help us create a better picture of the possible health effects on human beings. The results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects and may alter the hematological, biochemical, and immunologic parameters. However, many years of research with animals and clinical trials are required for this assessment. The use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which may promote cancer.

http://www.ncbi.nlm.nih.gov/pubmed/18989835

REV - Camara, M.; Marinho, C.; Guilam, M.; Nodari, R. 2009. Transgênicos: avaliação da possível (in)segurança alimentar através da produção cientifica. *História, Ciências, Saúde – Manguinhos*, Rio de Janeiro, v.16, n.3, p.669-681.

Identifica e analisa criticamente a produção científica brasileira, no campo da saúde pública, sobre os organismos geneticamente modificados, no que concerne à (in)segurança alimentar. Para tanto, realizou-se uma revisão bibliográfica nos portais do Scientific Eletronic Library Online (SciELO) e da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). Dos 716 trabalhos encontrados, apenas oito abordam a segurança alimentar dos transgênicos, através, principalmente, da exposição aos riscos e das incertezas desses produtos para a saúde e o meio ambiente. A principal conclusão do estudo refere-se ao fato de que os oito trabalhos analisados discursam, não sobre a segurança, mas sobre a insegurança dos alimentos geneticamente modificados.

Artigo completo disponível em http://www.scielo.br/scielo.php?pid=S0104-59702009000300006&script=sci_abstract&tlng=pt

REV - Magana-Gomez, J.; de la Barca, A. 2009. Risk assessment of genetically modified crops for nutrition and health. *Nutr Rev*, 67 (1).

The risk assessment of genetically modified (GM) crops for human nutrition and health has not been systematic. Evaluations for each GM crop or trait have been conducted using different feeding periods, animal models, and parameters. The most common result is that GM and conventional sources induce similar nutritional performance and growth in animals. However, adverse microscopic and molecular effects of some GM foods in different organs or tissues have been reported. Diversity among the methods and results of the risk assessments reflects the complexity of the subject. While there are currently no standardized methods to evaluate the safety of GM foods, attempts towards harmonization are on the way. More scientific effort is necessary in order to build confidence in the evaluation and acceptance of GM foods.

http://www.ncbi.nlm.nih.gov/pubmed/19146501

REV - Vendômois, J.; Cellier, D.; Vélot, C.; Clair, E.; Mesnage, R.; Seralini, G. 2010. Debate on GMOs health risks after statistical findings in regulatory tests. *International Journal Biological Science*, 6, 590–598.

We summarize the major points of international debate on health risk studies for the main commercialized edible GMOs. These GMOs are soy, maize and oilseed rape designed to contain new pesticide residues since they have been modified to be herbicide-tolerant (mostly to Roundup) or to produce mutated Bt toxins. The debated alimentary chronic risks may come from unpredictable insertional mutagenesis effects, metabolic effects, or from the new pesticide residues. The most detailed regulatory tests on the GMOs are three-month long feeding trials of laboratory rats, which are biochemically assessed. The tests are not compulsory, and are not independently conducted. The test data and the corresponding results are kept in secret by the companies. Our previous analyses of regulatory raw data at these levels, taking the representative examples of three GM maize NK 603, MON 810, and MON 863 led us to conclude that hepatorenal toxicities were possible, and that longer testing was necessary. Our study was criticized by the company developing the GMOs in question and the regulatory bodies, mainly on the divergent biological interpretations of statistically significant biochemical and physiological effects. We present the scientific reasons for the crucially different biological interpretations and also highlight the shortcomings in the experimental protocols designed by the company. The debate implies an enormous responsibility towards public health and is essential due to nonexistent traceability or epidemiological studies in the GMO-producing countries.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952409/

REV - Domingo, J.; Bordonaba, J. 2011. A literature review on the safety assessment of genetically modified plants. *Environment International*, 37. 734-42.

In recent years, there has been a notable concern on the safety of genetically modified (GM) foods/plants, an important and complex area of research, which demands rigorous standards. Diverse groups including consumers and environmental Non Governmental Organizations (NGO) have suggested that all GM foods/plants should be subjected to long-term animal feeding studies before approval for human consumption. In 2000 and 2006, we reviewed the information published in international scientific journals, noting that the number of references concerning human and animal toxicological/health risks studies on GM foods/plants was very limited. The main goal of the present review was to assess the current state-of-the-art regarding the potential adverse effects/safety assessment of GM plants for human consumption. The number of citations found in databases (PubMed and Scopus) has dramatically increased since 2006. However, new information on products such as potatoes, cucumber, peas or tomatoes, among others was not available. Corn/maize, rice, and soybeans were included in the present review. An equilibrium in the number research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was currently observed. Nevertheless, it should be noted that most of these studies have been conducted by biotechnology companies responsible of commercializing these GM plants. These findings suggest a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies. All this recent information is herein critically reviewed.

http://www.ncbi.nlm.nih.gov/pubmed/21296423

Maghari, B.; Ardekani, A. 2011. Genetically modified foods and social concerns. *Avicenna Journal of Medical Biotechnology*, 3(3): 109–117. PMCID: PMC3558185.

Biotechnology is providing us with a wide range of options for how we can use agricultural and commercial forestry lands. The cultivation of genetically modified (GM) crops on millions of *hectares* of lands and their injection into our food chain is a huge global genetic experiment involving all living beings. Considering the fast pace of new advances in production of genetically modified crops, consumers, farmers and policymakers worldwide are challenged to reach a consensus on a clear vision for the future of world food supply. The current food biotechnology debate illustrates the serious conflict between two groups: 1) Agri-biotech investors and their affiliated scientists who consider agricultural biotechnology as a solution to food shortage, the scarcity of environmental resources and weeds and pests infestations; and 2) independent scientists, environmentalists, farmers and consumers who warn that genetically modified food introduces new risks to food security, the environment and human health such as loss of biodiversity; the emergence of superweeds and superpests; the increase of antibiotic resistance, food allergies and other unintended effects. This article reviews major viewpoints which are currently debated in the food biotechnology sector in the world. It also lays the ground-work for deep debate on benefits and risks of Biotech-crops for human health, ecosystems and biodiversity. In this context, although some regulations exist, there is a need for continuous vigilance for all countries involved in producing genetically engineered food to follow the international scientific bio-safety testing guidelines containing reliable pre-release experiments and post-release track of transgenic plants to protect public health and avoid future environmental harm.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3558185/

REV - Zdziarski, I.; Edwards, J.; Carman, J.; Haynes, J. 2014. GM crops and the rat digestive tract: A critical review. *Environment International*, 73 (2014) 423–433.

The aim of this review is to examine the relationship between genetically modified (GM) crops and health, based on histopathological investigations of the digestive tract in rats. We reviewed published long-term feeding studies of crops containing one or more of three specific traits: herbicide tolerance via the EPSPS gene and insect resistance via cry1Ab or cry3Bb1 genes. These genes are commonly

> found in commercialised GM crops. Our search found 21 studies for nine (19%) out of the 47 crops approved for human and/or animal consumption. We could find no studies on the other 38 (81%) approved crops. Fourteen out of the 21 studies (67%) were general health assessments of the GM crop on rat health. Most of these studies (76%) were performed after the crop had been approved for human and/or animal consumption, with half of these being published at least nine years after approval. Our review also discovered an inconsistency in methodology and a lack of defined criteria for outcomes that would be considered toxicologically or pathologically significant. In addition, there was a lack of transparency in the methods and results, which made comparisons between the studies difficult. The evidence reviewed here demonstrates an incomplete picture regarding the toxicity (and safety) of GM products consumed by humans and animals. Therefore, each GM product should be assessed on merit, with appropriate studies performed to indicate the level of safety associated with them. Detailed guidelines should be developed which will allow for the generation of comparable and reproducible studies. This will establish a foundation for evidence-based guidelines, to better determine if GM food is safe for human and animal consumption.
>
> Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0160412014002669

Apesar das incertezas e das deficiências de informações relacionadas aos riscos para a saúde dos consumidores, cabe mencionar dois estudos epidemiológicos extremamente relevantes, inclusive por sua singularidade e aparente ineditismo. Trata-se – no limite de nosso conhecimento – dos dois únicos trabalhos desse tipo disponibilizados na literatura científica internacional. Ambos revelam indicativos de degradação na saúde da população dos EUA, ao longo das duas últimas décadas, associando este fenômeno à ampliação do cultivo de plantas GM naquele país. Por sua importância, e levando em conta os paralelos que permitem estabelecer, estes estudos revelam a necessidade de avaliações detalhadas, que busquem identificar ou negar a presença de relações de causalidade mais fortes do que aquelas identificadas por métodos de correlação.

Os estudos em questão contrariam diretamente a argumentação mais frequentemente repetida pelas empresas e demais promotores dessas biotecnologias: “após 20 anos consumindo plantas GM o mundo jamais percebeu qualquer problema de saúde a elas associado[53]”.

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[53] Teoricamente, tal afirmativa deveria se basear em algum tipo de estudo epidemiológico, estabelecendo comparação entre consumo de populações distintas e diversas, por longo prazo, levando em conta amplo leque de variáveis biológicas associadas ao estado de saúde geral das pessoas. Basicamente, o grupo teste deveria se alimentar fundamentalmente com produtos de origem transgênica, que seriam evitados no grupo controle. Este, idealmente, se subdividiria, com parte sendo alimentada com produtos da agricultura tradicional e parte com produtos oriundos da agricultura orgânica. Em que pese seu caráter básico e fundamental, após 20 anos de liberação das PGMs, as agências de saúde humana e animal ainda não se propuseram a realizar esse tipo de estudo. Reputamos os interesses relacionados à indisponibilidade desse estudo como razão para sua inexistência no conjunto da literatura científica especializada, em escala internacional.

A ausência de estudos epidemiológicos, que jamais poderia ser utilizada – por formadores de opinião ou por pesquisadores responsáveis –, como argumento ilustrativo para atestar inexistência de danos do tipo epidemiológico, é desmascarada com base nos trabalhos a seguir.

EPI - Shao, Q.; Chin, K. 2011. Survey of American food trends and the growing obesity epidemic. *Nutr Res Pract*, 5(3): 253-259.

The rapid rise in the incidence of obesity has emerged as one of the most pressing global public health issues in recent years. The underlying etiological causes of obesity, whether behavioral, environmental, genetic, or a combination of several of them, have not been completely elucidated. The obesity epidemic has been attributed to the ready availability, abundance, and overconsumption of high-energy content food. We determined here by Pearson's correlation the relationship between food type consumption and rising obesity using the loss-adjusted food availability data from the United States Department of Agriculture (USDA) Economic Research Services (ERS) as well as the obesity prevalence data from the Behavioral Risk Factor Surveillance System (BRFSS) and the National Health and Nutrition Examination Survey (NHANES) at the Centers for Disease Control and Prevention (CDC). Our analysis showed that total calorie intake and consumption of high fructose corn syrup (HFCS) did not correlate with rising obesity trends. Intake of other major food types, including chicken, dairy fats, salad and cooking oils, and cheese also did not correlate with obesity trends. However, our results surprisingly revealed that consumption of corn products correlated with rising obesity and was independent of gender and race/ethnicity among population dynamics in the U.S. Therefore, we were able to demonstrate a novel link between the consumption of corn products and rising obesity trends that has not been previously attributed to the obesity epidemic. This correlation coincides with the introduction of bioengineered corns into the human food chain, thus raising a new hypothesis that should be tested in molecular and animal models of obesity.

http://www.ncbi.nlm.nih.gov/pubmed/21779530

EPI - Swanson, N.; Leu, A.; Abrahamson, J.; Wallet, B. 2014. Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. *Jornal of Organic Systems*, 9(2).

A huge increase in the incidence and prevalence of chronic diseases has been reported in the United States (US) over the last 20 years. Similar increases have been seen globally. The herbicide glyphosate was introduced in 1974 and its use is accelerating with the advent of herbicide-tolerant genetically engineered (GE) crops. Evidence is mounting that glyphosate interferes with many metabolic processes in plants and animals and glyphosate residues have been detected in both. Glyphosate disrupts the endocrine system and the balance of gut bacteria, it damages DNA and is a driver of mutations that lead to cancer. In the present study, US government databases were searched for GE crop data, glyphosate application data and disease epidemiological data. Correlation analyses were then performed on a total of 22 diseases in these time-series data sets. The Pearson correlation coefficients are highly significant (< 10-5) between glyphosate applications and hypertension (R = 0.923), stroke (R = 0.925), diabetes prevalence (R = 0.971), diabetes incidence (R = 0.935), obesity (R = 0.962), lipoprotein metabolism disorder (R = 0.973), Alzheimer's (R = 0.917), senile dementia (R = 0.994), Parkinson's (R = 0.875), multiple sclerosis (R = 0.828), autism (R = 0.989), inflammatory bowel disease (R = 0.938), intestinal infections (R = 0.974), end stage renal disease (R = 0.975), acute kidney failure (R = 0.978), cancers of the thyroid (R = 0.988), liver (R = 0.960), bladder (R = 0.981), pancreas (R = 0.918), kidney (R = 0.973) and myeloid leukaemia (R = 0.878). The Pearson correlation coefficients are highly significant (< 10-4) between the percentage of

GE corn and soy planted in the US and hypertension (R = 0.961), stroke (R = 0.983), diabetes prevalence (R = 0.983), diabetes incidence (R = 0.955), obesity (R = 0.962), lipoprotein metabolism disorder (R = 0.955), Alzheimer's (R = 0.937), Parkinson's (R = 0.952), multiple sclerosis (R = 0.876), hepatitis C (R = 0.946), end stage renal disease (R = 0.958), acute kidney failure (R = 0.967), cancers of the thyroid (R = 0.938), liver (R = 0.911), bladder (R = 0.945), pancreas (R = 0.841), kidney (R = 0.940) and myeloid leukaemia (R = 0.889). The significance and strength of the correlations show that the effects of glyphosate and GE crops on human health should be further investigated.

Artigo completo disponível em http://www.organic-systems.org/journal/92/JOS_Volume-9_Number-2_Nov_2014-Swanson-et-al.pdf

1 Riscos para a saúde associados ao uso de plantas Bt

1.1 Possibilidades de interação entre proteínas Bt e células de mamíferos

A indústria das biotecnologias agrícolas argumenta que a ausência de riscos para a saúde humana e animal, no que diz respeito ao consumo de toxinas Bt, se deve ao fato de que essas proteínas apresentam estrita especificidade de ação. Condicionada à presença de receptores exclusivos do sistema digestivo de determinados insetos, e ausentes nos humanos e outros animais, essa característica eliminaria riscos. Além disso, afirmam que as proteínas seriam destruídas pela temperatura e pela acidez observadas no sistema digestivo de monogástricos e ruminantes.

Mais recentemente tais afirmativas deixaram de ser aceitas, embora continuem sendo repetidas. Além das já mencionadas falhas de especificidade para insetos, acumulam-se evidências de interações biológicas entre aquelas proteínas e células de mamíferos.

No item 1.1 da Parte 3 são expostas referências adicionais, apontando dificuldade de aceitação e mesmo de compreensão das razões que levam alguns estudiosos a manter, contra evidências, a argumentação de estrita especificidade de ação das toxinas Cry.

Vázquez-Padrón, R.; Gonzáles-Cabrera, J.; García-Tovar, C.; Neri-Bazan, L.; Lopéz- Revilla, R.; Hernández, M.; Moreno- Fierro, L.; de la Riva, G. 2000. Cry1Ac protoxin from *Bacillus thuringiensis* sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. *Biochem Biophys Res Comm*, 271, 54-58.

Bacillus thuringiensis (Bt), considered a safe insecticide, produces insecticidal proteins named Cry during sporulation, which possess exceptional immunological properties. In this work using an immunohistochemical test we demonstrated that Cry1Ac protoxin (pCry1Ac) binds to the mucosal surface of the mouse small intestine. Ligand blot assay allowed us to detect, under denaturing conditions, six pCry1Ac-binding polypeptides present in brush border membrane vesicles isolated from the small intestine. Moreover, this protein induced in situ temporal changes in the electrophysiological properties of the mouse jejunum. The data obtained indicate a possible interaction in vivo of Cry proteins with the animal bowel which could induce changes in the physiological status of the intestine.

http://www.ncbi.nlm.nih.gov/pubmed/10777680

Mesnage, R.; Clair, E.; Gress, S.; Then, C; Székács, A.; Séralini, G-E. 2012. Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. *Journal of Applied Toxicology*, DOI: 10.1002/jat.2712.

The study of combined effects of pesticides represents a challenge for toxicology. In the case of the new growing generation of genetically modified (GM) plants with stacked traits, glyphosate-based herbicides (like Roundup) residues are present in the Roundup-tolerant edible plants (especially corns) and mixed with modified Bt insecticidal toxins that are produced by the GM plants themselves. The potential side effects of these combined pesticides on human cells are investigated in this work. Here we have tested for the very first time Cry1Ab and Cry1Ac Bt toxins (10 ppb to 100 ppm) on the human embryonic kidney cell line 293, as well as their combined actions with Roundup, within 24 h, on three biomarkers of cell death: measurements of mitochondrial succinate dehydrogenase, adenylate kinase release by membrane alterations and caspase 3/7 inductions. Cry1Abcaused cell death from 100 ppm. For Cry1Ac, under such conditions, no effects were detected. The Roundup tested alone from 1 to 20 000 ppm is necrotic and apoptotic from 50 ppm, far below agricultural dilutions (50% lethal concentration 57.5 ppm). The only measured significant combined effect was that Cry1Ab and Cry1Ac reduced caspases 3/7 activations induced by Roundup; this could delay the activation of apoptosis. There was the same tendency for the other markers. In these results, we argue that modified Bt toxins are not inert on nontarget human cells, and that they can present combined side-effects with other residues of pesticides specific to GM plants.

Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2012/11/mesnage2011.pdf

Mezzomo, B.; Miranda-Vilela, A.; Freire, I.; Barbosa, L.; Portilho, F.; Lavaca, Z.; Grisolia, C. 2013. Hematotoxicity of *Bacillus thuringiensis* as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in swiss albino mice. *Journal of Hematology & Thromboembolic Diseases*, 1: 104. doi:10.4172/jhtd.1000104.

Formulated and sporulated cultures of Bacillus thuringiensis (Bt) have been widely used against insect pests, but after the advent of genetically modified plants expressing δ-endotoxins, the bioavailability of Cry proteins has been increased. For biosafety reasons their adverse effects should be studied, mainly for non-target organisms. Thus, we evaluated, in Swiss albino mice, the hematotoxicity and genotoxicity of four Bt spore-crystals genetically modified to express individually Cry1Aa, Cry1Ab, Cry1Ac or Cry2A, administered alone by gavage with a single dose of 27 mg/Kg, 136 mg/Kg or

270 mg/Kg, 24 h, 72 h or 7 days before euthanasia. Binary combinations of these four spore-crystal proteins were also assayed at 270 mg/Kg with a single administration 24 h before euthanasia. Control mice received filtered water or cyclophosphamide at 27 mg/kg. For hematotoxicity evaluations, blood samples were drawn by cardiac puncture and processed in a multiple automated hematology analyzer; for genotoxicity analyses, micronucleus test was carried out in mice bone marrow cells. Spore-crystal administrations provoked selective hematotoxicity for the 3 exposure times, particularly for erythroid lineage. A significant reduction in bone marrow cell proliferation demonstrated cytotoxic but not genotoxic effects. These effects persisted for all exposure times, becoming more evident at 7 days.Similar results were observed for binary combinations at 24 h, suggesting that further studies are required to clarify the mechanism involved in the hematotoxicity found in mice, and to establish the toxicological risks to non-target organisms, especially mammals, before concluding that these microbiological control agents are safe for mammals.

Artigo completo disponível em http://www.esciencecentral.org/journals/hematotoxicity-of-bacillus-thuringiensis-as-spore-crystal-strains-cry1aa-cry1ab-cry1ac-or-cry2aa-in-swiss-albino-mice-2329-8790.1000104.pdf

1.2 O destino das proteínas Bt (no organismo dos seres que as ingerem)

A indústria de biotecnologias agrícolas, bem como os órgãos de avaliação do risco, assumem que as proteínas Cry serão totalmente degradadas pelo sistema digestivo dos organismos animais (não conseguirão ultrapassar a barreira histológica gastrointestinal). Observações nesse sentido, obtidas com base em resultados de simulações de laboratório, têm sido interpretadas como suficientemente informativas para permitir dispensa de estudos mais completos. Assim, praticamente inexistem avaliações do risco examinando a presença e o efeito destas toxinas no organismo de animais superiores.

Entretanto, vários artigos mostram que as proteínas Cry podem sobreviver à digestão em animais e humanos, ultrapassando as barreiras naturais e mantendo parte significativa de suas atividades biológicas. Mesmo a barreira da placenta é ultrapassada por fragmentos dessas toxinas, que já foram relatadas como presentes no sistema circulatório de fetos e recém-nascidos.

Referências que mostram a circulação dessas toxinas entre artrópodes (insetos em especial) e micro-organismos do solo, bem como demonstram sua persistência ao longo de cadeias tróficas de agrossistemas, podem ser encontradas na Parte 3 item 1.3 deste livro.

Chowdhury, E.; Kuribara, H.; Hino, A.; Sultana, P.; Mikami, O.; Shimada, N.; Guruge, K.; Saito, M.; Nakajima, Y. 2003. Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. *Journal of Animal Science*, 81:2546-2551.

Genetically modified corn has been approved as an animal feed in several countries, but information about the fate of genetically modified DNA and protein in vivo is insufficient. Genetically modified corn Bt11 is developed by inserting a recombinant DNA sequence encoding insecticidal Cry1Abprotein from Bacillus thuringiensis subsp. kurstaki. We examined the presence of corn intrinsic and recombinant cry1Ab gene by PCR, and theCry1Ab protein by immunological tests in the gastrointestinal contents of five genetically modified corn Bt11-fed and five nongenetically modified corn-fed pigs. Fragments of corn zein (242 bp), invertase (226 bp) and of ribulose-1,5-bisphosphate carboxylase/ oxygenase genes (1,028 bp) were detected in the gastrointestinal contents of both Bt11 and nongenetically modified corn-fed pigs. Fragments of recombinant cry1Ab gene (110 bp and 437 bp) were detected in the gastrointestinal contents of the Bt11-fed pigs but not in the control pigs. Neither corn intrinsic nor cry1Ab gene fragments were detected in the peripheral blood by PCR. The gastrointestinal contents were positive for Cry1Ab protein by ELISA, immunochromatography, and immunoblot; however, these methods did not work for blood and precluded conclusions about any potential absorption of the protein. These results suggest that ingested corn DNA and Cry1Ab protein were not totally degraded in the gastrointestinal tract, as shown by their presence in a form detectable by PCR or immunological tests.

http://www.ncbi.nlm.nih.gov/pubmed/14552382

Lutz, B.; Wiedemann, S.; Einspanier, R; Mayer, J.; Albrecht, C. 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. *J Agric Food Chem*, 53:1453–1456.

Immunoblotting assays using commercial antibodies were established to investigate the unexpected persistence of the immunoactive Cry1Ab protein in the bovine gastrointestinal tract (GIT) previously suggested by enzyme-linked immunosorbent assay (ELISA). Samples of two different feeding experiments in cattle were analyzed with both ELISA and immunoblotting methods. Whereas results obtained by ELISA suggested that the concentration of the Cry1Ab protein increased during the GIT passage, the immunoblotting assays revealed a significant degradation of the protein in the bovine GIT. Samples showing a positive signal in the ELISA consisted of fragmented Cry1Ab protein of approximately 17 and 34 kDa size. Two independent sets of gastrointestinal samples revealed the apparent discrepancy between the results obtained by ELISA and immunoblotting, suggesting that the antibody used in the ELISA reacts with fragmented yet immunoactive epitopes of the Cry1Ab protein. It was concluded that Cry1Ab protein is degraded during digestion in cattle. To avoid misinterpretation, samples tested positive for Cry1Ab protein by ELISA should be reassessed by another technique.

http://www.ncbi.nlm.nih.gov/pubmed/15740023

Guimaraes, V.; Drumare, M.; Lereclus, D.; Gohar, M.; Lamourette, P.; Nevers, M.; Vaisanen-Tunkelrott, M.; Bernard, H.; Guillon, B.; Créminon, C.; Wal J.; Adel-Patient, K. 2010. *In vitro* digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity. *J Agric Food Chem*, 58(5): 3222-3231.

A pepsin resistance test performed at pH 1.2 and with high pepsin to protein ratio is one of the steps of the weight-of-evidence approach used for assessment of allergenicity of new proteins. However, the use of other in vitro digestibility tests, performed in more physiologically relevant conditions and in combination with immunological assays so as to increase the value of the information gained from the studies of stability of a novel protein to digestion for the overall allergenicity assessment, has been proposed. This study then aimed to investigate the stability to digestion of Cry1Abprotoxin and toxin, insecticidal proteins expressed in genetically modified crops, using simulated gastric fluid (SGF) at different pH values and pepsin-to-substrate ratios, in the presence or absence of physiological surfactant phosphatidylcholine (PC). Electrophoresis and immunoblot patterns and residual immunoreactivity of digesta were analyzed. Although Cry1Ab protoxin is extensively degraded at pH 1.2 with high pepsin-to-protein ratio, it is only slightly degraded at pH 2.0 and conserved its immunoreactivity. Furthermore, Cry1Ab proteins were demonstrated to be stable in a more physiologically relevant in vitro digestibility test (pH 2.5, pepsin-to-substrate ratio 1:20 (w/w) with PC). Factors such as pH, SGF composition, and pepsin-to-substrate ratio then greatly influence the digestion of Cry1Ab proteins, confirming that new and more physiologically relevant in vitro digestibility tests should be also considered to study the relationship between the resistance of a protein to digestion and its allergenicity.

http://www.ncbi.nlm.nih.gov/pubmed/20136083

Aris, A; Leblanc, S. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, 31(4): 528-33. doi: 10.1016/j.reprotox.

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

http://www.ncbi.nlm.nih.gov/pubmed/21338670

1.3 O potencial alergênico das plantas Bt

O risco de problemas alérgicos decorrentes do contato com as toxinas Cry exige esforços concentrados adicionais ao realizado até este momento. Não há consenso científico. A literatura especializada constata presença de reações imunológicas ou alergênicas em estudos realizados com animais e seres humanos. A literatura também informa que tais reações se associam à presença de proteínas Bt e de outros componentes observados (inesperados por ocasião do processo de inserção do transgene) nas PGMs.

Também seriam frequentes reações imunológicas de baixo impacto, incapazes de provocar – de imediato – reações alergênicas. Biologicamente mais difusas, ainda que perceptíveis ao consumidor, essas reações podem trazer implicações de longo prazo, de momento desconhecidas. Sabe-se que reações imunológicas continuadas, se mantidas ativas pelo organismo ao longo do tempo, podem vir a resultar em reações alergênicas e/ou inflamatórias, entre outras. Além disso, por efeitos sinérgicos e reações retroalimentadas, reações imunológicas simples contra determinadas moléculas podem vir a se transformar em reações alergênicas complexas, contra outras moléculas.

Vázquez-Padrón, R.; Moreno-Fierros, L.; Neri-Bazan, L.; de la Riva, G.; Lopez-Revilla, R. 1999. Intragastric and intra-peritoneal administration of Cry1Ac protoxin from *Bacillus thuringiensis* induces systemic and mucosal antibody responses in mice. *Life Sciences*, 64 (21): 1897-1912.

The spore-forming soil bacterium Bacillus thuringiensis produces parasporal inclusion bodies composed by delta-endotoxins also known as Cry proteins, whose resistance to proteolysis, stability in highly alkaline pH and innocuity to vertebrates make them an interesting candidate to carrier of relevant epitopes in vaccines. The purpose of this study was to determine the mucosal and systemic immunogenicity in mice of Cry1Ac protoxin from B. thuringiensis HD73. Crystalline and soluble forms of the protoxin were administered by intraperitoneal or intragastric route and anti-Cry1Acantibodies of the major isotypes were determined in serum and intestinal fluids. The two forms of Cry1Ac protoxin administered by intraperitoneal route induced a high systemic antibody response, however, only soluble Cry1Ac induced a mucosal response via intragastric. Serum antibody levels were higher than those induced by cholera toxin. Systemic immune responses were attained with doses of soluble Cry1Ac ranging from 0.1 to 100 microg by both routes, and the maximal effect was obtained with the highest doses. High anti-Cry1Ac IgG antibody levels were detected in the large and small intestine fluids from mice receiving the antigen via i.p. These data indicate that Cry1Ac is a potent systemic and mucosal immunogen.

http://www.ncbi.nlm.nih.gov/pubmed/10353588

Bernstein, I.; Bernstein, J.; Miller, M.; Tierzieva, S.; Bernstein, D.; Lummus, Z.; Selgrade, M.; Doerfler, D.; Seligy, V. 1999. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environmental Health Perspectives*, 107(7): 575-582.

Although health risks to pesticides containing Bacillus thuringiensis (Bt) have been minimal, the potential allergenicity of these organisms has not been evaluated. Therefore, a health survey was conducted in farm workers before and after exposure to Bt pesticides. Farm workers who picked vegetables that required Bt pesticide spraying were evaluated before the initial spraying operation (n = 48) and 1 and 4 months after (n = 32 and 20, respectively). Two groups of low- (n = 44) and medium- (n = 34) exposure workers not directly exposed to Bt spraying were also assessed. The investigation included questionnaires, nasal/mouth lavages, ventilatory function assessment, and skin tests to indigenous aeroallergens and to a variety of Bt spore and vegetative preparations. To authenticate exposure to the organism present in the commercial preparation, isolates from lavage specimens were tested for Bt genes by DNA-DNA hybridization. Humoral immunoglobulin G (IgG) and immunoglobulin E (IgE) antibody responses to spore and vegetative Bt extracts were assayed. There was no evidence of occupationally related respiratory symptoms. Positive skin-prick tests to several spore extracts were seen chiefly in exposed workers. In particular, there was a significant ($p < 0.05$) increase in the number of positive skin tests to spore extracts 1 and 4 months after exposure to

Bt spray. The number of positive skin test responses was also significantly higher in high ($p < 0.05$) than in low- or medium-exposure workers. The majority of nasal lavage cultures from exposed workers was positive for the commercial Bt organism, as demonstrated by specific molecular genetic probes. Specific IgE antibodies were present in more high-exposure workers ($p < 0.05$) than in the low and medium groups. Specific IgG antibodies occurred more in the high ($p < 0.05$) than in the low-exposure group. Specific IgG and IgE antibodies to vegetative organisms were present in all groups of workers. Exposure to Bt sprays may lead to allergic skin sensitization and induction of IgE and IgG antibodies, or both.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1566654/

Vázquez, R.; Moreno-Fierros, L.; Neri-Bazan, L.; De La Riva, G.; Lopez-Revilla, R. 1999. *Bacillus thuringiensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. *Scandinavian Journal of Immunology*, 49(6): 578-84.

Recently we demonstrated that recombinant Cry1Ac protoxin from Bacillus thuringiensis is a potent systemic and mucosal immunogen. In this study we compared the adjuvant effects of Cry1Ac and cholera toxin (CT) for the hepatitis B surface antigen (HBsAg) and bovine serum albumin (BSA). The antibody responses of intestinal secretions and serum were determined by ELISA in Balb/c mice immunized through the intragastric (IG) or intraperitoneal (IP) routes. When HBsAg was administered via IG, the anti-HBsAg intestinal response was not enhanced by either Cry1Ac or CT, whereas via IP Cry1Ac increased the anti-HBsAg intestinal immunoglobulin (Ig) G response and CT increased the intestinal IgA and IgM responses. Serum anti-BSA antibodies increased when BSA was co-administered with CT or Cry1Ac by both routes. Cholera toxin and Cry1Ac co-administered via IP increased the IgG anti-BSA response in fluid of the large intestine and CT also increased the IgA and IgM responses slightly. When co-administered via IP, CT and Cry1Ac did not affect the IgG anti-BSA response of the small intestine significantly. We conclude that Cry1Ac is a mucosal and systemic adjuvant as potent as CT which enhances mostly serum and intestinal IgG antibody responses, especially at the large intestine, and its effects depend on the route and antigen used. These features make Cry1Ac of potential use as carrier and/or adjuvant in mucosal and parenteral vaccines.

http://www.ncbi.nlm.nih.gov/pubmed/10354369

Vázquez-Padrón, R.; Moreno-Fierros, l.; Neri-Bazán, l.; Martínez-Gil, A.; de-la-Riva, A.; López-Revilla, R. 2000. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from *Bacillus thuringiensis* HD 73 in mice. *Brazilian Journal of Medical and Biological Research*, 33, 147-155.

The present paper describes important features of the immune response induced by the Cry1Ac protein from Bacillus thuringiensis in mice. The kinetics of induction of serum and mucosal antibodies showed an immediate production of anti-Cry1Ac IgM and IgG antibodies in serum after the first immunization with the protoxin by either the intraperitoneal or intragastric route. The antibody fraction in serum and intestinal fluids consisted mainly of IgG1. In addition, plasma cells producing anti-Cry1Ac IgG antibodies in Peyer's patches were observed using the solid-phase enzyme-linked immunospot (ELISPOT). Cry1Ac toxin administration induced a strong immune response in serum but in the small intestinal fluids only anti-Cry1AcIgA antibodies were detected. The data obtained in the present study confirm that the Cry1Ac protoxin is a potent immunogen able to induce a specific immune response in the mucosal tissue, which has not been observed in response to most other proteins.

http://www.ncbi.nlm.nih.gov/pubmed/10657055

Moreno-Fierros, l.; Garcíaa, N.; Gutiérrez, R.; López-Revilla, R.; Vázquez-Padrón, R. 2000. Intranasal, rectal and intraperitoneal immunization with protoxin Cry1Ac from *Bacillus thuringiensis* induces compartmentalized serum, intestinal, vaginal and pulmonary immune responses in Balb/c mice. *Microbes and Infection*, 2, 2000, 885–890.

Recently we discovered that the Cry1Ac protoxin of Bacillus thuringiensis administered to Balb/c mice intraperitoneally (i.p.) or intragastrically is a systemic and intestinal immunogen as potent as cholera toxin. To further characterize the mucosal immunogenicity of Cry1Ac we additionally tried the intranasal (i.n.) and rectal routes and used enzyme-linked immunoassays to determine anti-Cry1Ac antibody responses in the serum as well as invaginal and tracheobronchial washes and in the fluids of the large and the small intestine. Immunization by the i.p., i.n. and rectal routes induced IgM, IgG and IgA antibodies in all the mucosal surfaces analyzed, but the magnitude and predominant isotype of each response depended on the route used and the mucosal site analyzed. These data extend our findings on the striking mucosal immunogencity of Cry1Ac and provide additional evidence on the compartmentalization of the mucosal immune system.

http://www.ncbi.nlm.nih.gov/pubmed/10962271

Sagstad, A.; Sanden, M.; Haugland, O.; Hansen A.; Olsvik P.; Hemre G. 2007. Evaluation of stress and immune-response biomarkers in Atlantic salmon, Salmo salar L., fed different levels of genetically modified maize (Bt maize), compared with its near-isogenic parental line and a commercial suprex maize. *Journal of Fish Diseases*, 30: 201–212.

The present study was designed to evaluate if genetically modified (GM) maize (Bt maize, event MON810) compared with the near-isogenic non-modified (nGM) maize variety, added as a starch source at low or high inclusions, affected fish health of post-smolt Atlantic salmon, Salmo salar L. To evaluate the health impact, selected stress- and immune-response biomarkers were quantified at the gene transcript (mRNA) level, and some also at the protein level. The diets with low or high inclusions of GM maize, and its near-isogenic nGM parental line, were compared to a control diet containing GM-free suprex maize (reference diet) as the only starch source. Total superoxide dismutase (SOD) activity in liver and distal intestine was significantly higher in fish fed GM maize compared with fish fed nGM maize and with the reference diet group. Fish fed GM maize showed significantly lower catalase (CAT) activity in liver compared with fish fed nGM maize and to the reference diet group. In contrast, CAT activity in distal intestine was significantly higher for fish fed GM maize compared with fish fed reference diet. Protein level of heat shock protein 70 (HSP70) in liver was significantly higher in fish fed GM maize compared with fish fed the reference diet. No diet-related differences were found in normalized gene expression of SOD, CAT or HSP70 in liver or distal intestine. Normalized gene expression of interleukin-1 beta in spleen and head-kidney did not vary significantly between diet groups. Interestingly, fish fed high GM maize showed a significantly larger proportion of plasma granulocytes, a significantly larger sum of plasma granulocyte and monocyte proportions, but a significantly smaller proportion of plasma lymphocytes, compared with fish fed high nGM maize. In conclusion, Atlantic salmon fed GM maize showed some small changes in stress protein levels and activities, but none of these changes were comparable to the normalized gene expression levels analysed for these stress proteins. GM maize seemed to induce significant changes in white blood cell populations which are associated with an immune response.

http://www.ncbi.nlm.nih.gov/pubmed/17394522

Finamore, A.; Roselli, M.; Britti, S.; Monastra, G.; Ambra, R.; Turrini, A.; Mengheri, E. 2008. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. *Journal of Agricultural and Food Chemistry*, 56, 11533-11539.

This study evaluated the gut and peripheral immune response to genetically modified (GM) maize in mice in vulnerable conditions. Weaning and old mice were fed a diet containing MON810 or its parental control maize or a pellet diet containing a GM-free maize for 30 and 90 days. The immunophenotype of intestinal intraepithelial, spleen, and blood lymphocytes of control maize fed mice was similar to that of pellet fed mice. As compared to control maize, MON810 maize induced alterations in the percentage of T and B cells and of CD4(+), CD8(+), gammadeltaT, and alphabetaT subpopulations of weaning and old mice fed for 30 or 90 days, respectively, at the gut and peripheral sites. An increase of serum IL-6, IL-13, IL-12p70, and MIP-1beta after MON810 feeding was also found. These results suggest the importance of the gut and peripheral immune response to GM crop ingestion as well as the age of the consumer in the GMO safety evaluation.

Artigo completo disponível em http://www.giovannimonastra.info/documenti_pdf/Monastra_J_Agr_Food_Chem_2.pdf

Kroghsbo, S.; Madsen, C.; Poulsen, M.; Schrødera, M.; Kvista, P.; Taylor, M.; Gatehoused, A.; Shu, Q.; Knudsena, I. 2008. Immunotoxicological studies of genetically modified rice expressing PHA-E lectin or Bt toxin in Wistar rats. *Toxicology*, 245(1-2): 24-34.

As part of the SAFOTEST project the immunmodulating effect of Cry1Ab protein from *Bacillus thuringiensis*(Bt) and PHA-E lectin from kidney bean (*Phaseolus vulgaris* erythroagglutinin) was examined in 28- and 90-day feeding studies in Wistar rats. PHA-E lectin was chosen as positive control. Rats were fed control rice, transgenic rice expressing Cry1Ab protein or PHA-E lectin, or transgenic rice spiked with the purified recombinant protein. Total immunoglobulin levels, mitogen-induced cell proliferation, T-dependent antibody response to sheep red blood cells and the antigen-specific antibody response in serum were examined at the end of the studies. A dose-dependent increase in mesenteric lymph node weight and total immunoglobulin A was seen when feeding PHA-E transgenic rice alone or spiked with 0.1% purified PHA-E lectin for 90 days indicating a local effect of PHA-E in the intestine. No adverse effects of Cry1Ab protein were found. An anti-PHA-E and anti-Cry1Ab antibody response was induced both after inhalation (control groups) and after inhalation/ingestion (groups fed recombinant protein alone or together with transgenic rice). In conclusion, only PHA-E lectin was found to have an immunomodulating effect when feeding rats for 90 days with approximately 70 mg PHA-E/kg bodyweight per day. As both PHA-E lectin and Cry1Ab protein were capable of inducing an antigen-specific antibody response it is important to make careful considerations when designing future animal studies to avoid intake of proteins from the other groups by inhalation as well as to examine the sensitization and elicitation potential of 'foreign' proteins before introduction to the world market.

http://www.sciencedirect.com/science/article/pii/S0300483X07008281

Guimaraes, V.; Drumare, M.; Lereclus, D.; Gohar, M.; Lamourette, P.; Nevers, M.; Vaisanen-Tunkelrott, M.; Bernard, H.; Guillon, B.; Créminon, C.; Wal, J.; Adel-Patient, K. 2010. *In vitro* digestion of Cry1Ab proteins and analysis of the impact on their immunoreactivity. *J Agric Food Chem*, 58(5): 3222- 3231.

A pepsin resistance test performed at pH 1.2 and with high pepsin to protein ratio is one of the steps of the weight-of-evidence approach used for assessment of allergenicity of new proteins. However, the use of other in vitro digestibility tests, performed in more physiologically relevant conditions and in combination with immunological assays so as to increase the value of the information gained from the studies of stability of a novel protein to digestion for the overall allergenicity assessment, has been proposed. This study then aimed to investigate the stability to digestion of Cry1Abprotoxin and toxin, insecticidal proteins expressed in genetically modified crops, using simulated gastric fluid (SGF) at different pH values and pepsin-to-substrate ratios, in the presence or absence of physiological surfactant phosphatidylcholine (PC). Electrophoresis and immunoblot patterns and residual immunoreactivity of digesta were analyzed. Although Cry1Ab protoxin is extensively degraded at pH 1.2 with high pepsin-to-protein ratio, it is only slightly degraded at pH

2.0 and conserved its immunoreactivity. Furthermore, Cry1Ab proteins were demonstrated to be stable in a more physiologically relevant in vitro digestibility test (pH 2.5, pepsin-to-substrate ratio 1:20 (w/w) with PC). Factors such as pH, SGF composition, and pepsin-to-substrate ratio then greatly influence the digestion of Cry1Ab proteins, confirming that new and more physiologically relevant in vitro digestibility tests should be also considered to study the relationship between the resistance of a protein to digestion and its allergenicity.

http://www.ncbi.nlm.nih.gov/pubmed/20136083

Walsh, M.; Buzoianu, S.; Gardiner, G.; Rea, M.; Gelencsér, E.; Jánosi, A.; Epstein, M.; Ross, R.; Lawlor, P. 2011. Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. *PLOS ONE*, 6(11): e27177, doi:10.1371/journal.pone.0027177.

We assessed the effect of short-term feeding of genetically modified (GM: Bt MON810) maize on immune responses and growth in weanling pigs and determined the fate of the transgenic DNA and protein in-vivo. Pigs were fed a diet containing 38.9% GM or non-GM isogenic parent line maize for 31 days. We observed that IL-12 and IFNγ production from mitogenic stimulated peripheral blood mononuclear cells decreased ($P<0.10$) following 31 days of GM maize exposure. While Cry1Ab-specific IgG and IgA were not detected in the plasma of GM maize-fed pigs, the detection of the cry1Ab gene and protein was limited to the gastrointestinal digesta and was not found in the kidneys, liver, spleen, muscle, heart or blood. Feeding GM maize to weanling pigs had no effect on growth performance or body weight. IL-6 and IL-4 production from isolated splenocytes were increased ($P<0.05$) in response to feeding GM maize while the proportion of CD4(+) T cells in the spleen decreased. In the ileum, the proportion of B cells and macrophages decreased while the proportion of CD4(+) T cells increased in GM maize-fed pigs. IL-8 and IL-4 production from isolated intraepithelial and lamina propria lymphocytes were also increased ($P<0.05$) in response to feeding GM maize. In conclusion, there was no evidence of cry1Ab gene or protein translocation to the organs and blood of weaning pigs. The growth of pigs was not affected by feeding GM maize. Alterations in immune responses were detected; however, their biologic relevance is questionable.

Artigo completo disponível em http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177

Gu, J.; Krogdahl, Å.; Sissener, N.; Kortner, T.; Gelencser, E.; Hemre, G.; Bakke, A. 2013. Effects of oral Bt-maize (MON810) exposure on growth and health parameters in normal and sensitised Atlantic salmon, Salmo salar L. *Br J Nutr*, 109(8):1408-23.

Responses to GM maize Bt-maize, MON810) expressing Cry1Ab protein from the soil bacterium Bacillus thuringiensis (Bt) in diets for both normal and immune-sensitised (with soyabean meal (SBM)-induced enteropathy) post-smolt Atlantic salmon were investigated following 33 and 97 d of exposure. Triplicate tanks of salmon were fed one of four diets, all containing 20% whole-kernel meal maize, either Bt-maize or its near-isogenic maternal line, without or with 15% extracted SBM inclusion. The fish fed Bt-maize utilised the feed less efficiently, as revealed by lower protein and mineral digestibilities and lower lipid and energy retention efficiencies. Higher intestinal weight, as well as increased interferon-γ and decreased sodium-glucose co-transporter mRNA expression, and a transient increase in T-helper cell presence, as measured by cluster of differentiation 4 (CD4) protein in the distal intestine (DI), may partly explain the lower nutrient digestibilities and retentions. The Bt-maize seemed to potentiate oxidative cellular stress in the DI of immune-sensitised fish, as indicated by increases in superoxide dismutase and heat shock protein 70 mRNA expression. The data suggest that Cry1Ab protein or other antigens in Bt-maize have local immunogenic effects in salmon DI. No systemic immune responses could be detected, as indicated by haematology, differential leucocyte counts, plasma clinical chemistry, as well as absence of Cry1Ab-specific antibodies and Cry1Ab protein in plasma. The responses to Bt-maize observed in the present study differed from results from earlier studies in salmon and other animals fed the same event Bt-maize. Longer-term experiments and more in-depth studies on intestinal physiology and immune responses are needed to evaluate health implications.

http://www.ncbi.nlm.nih.gov/pubmed/23182224

1.4 Riscos toxicológicos associados ao consumo de plantas Bt

Apesar de inseridas em parte significativa das cadeias agroalimentares animais e humanas desde uma década, o debate a respeito de riscos toxicológicos associados ao consumo de plantas Bt permanece inconcluso. A comunidade científica diverge quanto à dimensão dos possíveis problemas e à validade dos indícios que apontam sua inexistência.

Estudos recentes acumulam evidências de toxicidade e danos hepático-renais em animais que consomem plantas transgênicas Bt por longo prazo. Outros[54] estudos, quando identificam diferenças estatisticamente significativas, passam a interpretá-las como biologicamente irrelevantes, descartando sua importância como indicativas de riscos para o consumo. Em ambos os casos, uma abordagem precavida recomendaria o desenvolvimento de novos e mais detalhados ensaios, previamente a qualquer conclusão relativamente à biossegurança dessas PGMs.

Séralini, G-E.; Cellier, D.; de Vendomois, J. 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Archives Environmental Contamination and Toxicology*, 52, 596-602.

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[54] Tais como Hammond et al., 2006 (Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. *Food Chem Toxicol.* 2006, 44:1092-1099); Hammond et al., 2006b (Results of a 90-day safety assurance study with rats fed grain from corn rootworm-protected corn. *Food Chem Toxicol.* 2006, 44:147-160); Trabalza-Marinucci et al., 2008 (A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. *Livestock Science*. 2008; 113(2): 178–190); ou ainda Buzoianu et al., 2013 (Transgenerational effects of feeding genetically modified maize to nulliparous sows and offspring on offspring growth and health. *J Anim Sci*. 2013 Jan;91(1):318-30).

Health risk assessment of genetically modified organisms (GMOs) cultivated for food or feed is under debate throughout the world, and very little data have been published on mid- or long-term toxicological studies with mammals. One of these studies performed under the responsibility of Monsanto Company with a transgenic corn MON863 has been subjected to questions from regulatory reviewers in Europe, where it was finally approved in 2005. This necessitated a new assessment of kidney pathological findings, and the results remained controversial. An Appeal Court action in Germany (Münster) allowed public access in June 2005 to all the crude data from this 90-day rat-feeding study. We independently re-analyzed these data. Appropriate statistics were added, such as a multivariate analysis of the growth curves, and for biochemical parameters comparisons between GMO-treated rats and the controls fed with an equivalent normal diet, and separately with six reference diets with different compositions. We observed that after the consumption of MON863, rats showed slight but dose-related significant variations in growth for both sexes, resulting in 3.3% decrease in weight for males and 3.7% increase for females. Chemistry measurements reveal signs of hepatorenal toxicity, marked also by differential sensitivities in males and females. Triglycerides increased by 24-40% in females (either at week 14, dose 11% or at week 5, dose 33%, respectively); urine phosphorus and sodium excretions diminished in males by 31-35% (week 14, dose 33%) for the most important results significantly linked to the treatment in comparison to seven diets tested. Longer experiments are essential in order to indicate the real nature and extent of the possible pathology; with the present data it cannot be concluded that GM corn MON863 is a safe product.

Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2012/11/seralini.new_.an_.2007.pdf

Aysun, K.; Akay, M. 2008. A three generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. *Food and Chemical Toxicology*, 46, 1164–1170.

For the last ten years, in accordance with the increased use of genetically modified (GM) foods for human and livestock, a large number of feeding studies have been carried out. However, the evidence is still far from proving whether the long-term consumption of GM foods poses a possible danger for human or animal health. Therefore, this study was designed to evaluate the effects of transgenic corn on the rats that were fed through three generations with either GM corn or its conventional counterpart. Tissue samples of stomach, duodenum, liver and kidney were obtained for histopathological examinations. The average diameter of glomeruli, thickness of renal cortex and glomerular volume were calculated and number of affected animals/number of examined animals for liver and kidney histopathology were determined. Amounts of urea, urea nitrogen, creatinine, uric acid, total protein, albumin and globulin were determined; enzyme activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyltransferase, creatine kinase and amylase were measured in serum samples. No statistically significant differences were found in relative organ weights of rats within groups but there were some minimal histopathological changes in liver and kidney. Changes in creatinine, total protein and globulin levels were also determined in biochemical analysis.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/webfm/plataforma/threegenerationstudyinvestigation.pdf

Vendômois, J.; Roullier, F.; Cellier, D.; Seralini, G-E. 2009. A comparison of the effects of three GM corn varieties on mammalian health. *International Journal of Biological Sciences*, 5, 706–726.

We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been modified to be tolerant to the broad spectrum herbicide Roundup and thus contains residues of this formulation. MON 810 and MON 863 are engineered to synthesize two different Bt toxins used as insecticides. Approximately 60 different biochemical parameters were classified per organ and measured in serum and urine after 5 and 14 weeks of feeding. GM maize-fed rats were compared first to their respective isogenic or parental non-GM equivalent control groups. This was followed by comparison to six reference groups, which had consumed various other non-GM maize varieties. We applied nonparametric methods, including multiple pairwise comparisons with a False Discovery Rate approach. Principal Component Analysis allowed the investigation of scattering of different factors (sex, weeks of feeding, diet, dose and group). Our analysis clearly reveals for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also noticed in the heart, adrenal glands, spleen and haematopoietic system. We conclude that these data highlight signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM corn. In addition, unintended direct or indirect metabolic consequences of the genetic modification cannot be excluded.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2793308/

Séralini, G-E.; Vendômois, J.; Cellier, D.; Sultan, C.; Buiatti, M.; Gallagher, L.; Antoniou, M.; Dronamraju, K. 2009. How subchronic and chronic health effects can be neglected for GMOs, pesticides or chemicals. *International Journal of Biological Sciences*, 5:438-443.

Chronic health effects are increasing in the world such as cancers, hormonal, reproductive, nervous, or immune diseases, even in young people. During regulatory toxicological subchronic tests to prevent these on mammalian health, prior commercialization of chemicals, including pesticides and drugs, or GMOs, some statistically significant findings may be revealed. This discussion is about the need to investigate the relevant criteria to consider those as biologically significant. The sex differences and the non linear dose or time related effects should be considered in contrast to the claims of a Monsanto-supported expert panel about a GMO, the MON 863 Bt maize, but also for pesticides or drugs, in particular to reveal hormone-dependent diseases and first signs of toxicities.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2706426/

Adel-Patient, K.; Guimaraes, V.; Paris, A.; Drumare, M.; Ah-Leung, S.; Lamourette, P.; Nevers, M.; Canlet, C.; Molina, J.; Bernard, H.; Creminon, C.; Wal, J. 2011. Immunological and metabolomic impacts of administration of Cry1Ab protein and MON 810 maize in mouse. *PLOS ONE*, 6(1): e16346. doi:10.1371/journal.pone.0016346.

We have investigated the immunological and metabolomic impacts of Cry1Ab administration to mice, either as a purified protein or as the Cry1Ab-expressing genetically modified (GM) MON810 maize. Humoral and cellular specific immune responses induced in BALB/cJ mice after intra-gastric (i.g.) or intra-peritoneal (i.p.) administration of purified Cry1Ab were analyzed and compared with those induced by proteins of various immunogenic and allergic potencies. Possible unintended effects of the genetic modification on the pattern of expression of maize natural allergens were studied using IgE-immunoblot and sera from maize-allergic patients. Mice were experimentally sensitized (i.g. or i.p. route) with protein extracts from GM or non-GM maize, and then anti-maize proteins and anti-Cry1Ab-induced immune responses were analyzed. In parallel, longitudinal metabolomic studies were performed on the urine of mice treated via the i.g. route. Weak immune responses were observed after i.g. administration of the different proteins. Using the i.p. route, a clear Th2 response was observed with the known allergenic proteins, whereas a mixed Th1/Th2 immune response was observed with immunogenic protein not known to be allergenic and with Cry1Ab. This then reflects protein immunogenicity in the BALB/c Th2-biased mouse strain rather than allergenicity. No difference in natural maize allergen profiles was evidenced between MON810 and its non-GM comparator. Immune responses against maize proteins were quantitatively equivalent in mice treated with MON810 vs the non-GM counterpart and no anti-Cry1Ab-specific immune response was detected in mice that received MON810. Metabolomic studies showed a slight "cultivar" effect, which represented less than 1% of the initial metabolic information. Our results confirm the immunogenicity of purified Cry1Ab without evidence of allergenic potential. Immunological and metabolomic studies revealed slight differences in mouse metabolic profiles after i.g. administration of MON810 vs its non-GM counterpart, but no significant unintended effect of the genetic modification on immune responses was seen.

Artigo completo disponível em http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0016346

Gab-Alla, A.; El-Shamei, Z.; Shatta, A.; Moussa, E.; Rayan, A. 2012. Morphological and biochemical changes in male rats fed on genetically modified corn (Ajeeb YG). *Journal of American Science*, 8(9) (152).

This study was designed to evaluate the safety of genetically modified (GM) corn (Ajeeb YG). Corn grains from Ajeeb YG or its control (Ajeeb) were incorporated into rodent diets at 30% concentrations administered to rats (n= 10/group) for 45 and 91 days. An additional negative control group of rats (n= 10/group) was fed AIN93G diets. General conditions were observed daily, total body weights were recorded weekly. At the termination of the study periods, some visceral organs (heart, liver, kidneys, testes and spleen) and serum biochemistry were measured. The data showed several statistically significant differences in organs/body weight and serum biochemistry between the rats fed on GM and/or Non-GM corn and the rats fed on AIN93G diets. In general, GM corn sample caused several changes by increase or decrease organs/body weight or serum biochemistry values. This indicates potential adverse health/toxic effects of GM corn and further investigations still needed.

Artigo completo disponível em http://www.jofamericanscience.org/journals/am-sci/am0809/152_11742am0809_1117_1123.pdf

El-Shamei, Z.; Gab-Alla, A.; Shatta, A.; Moussa, E.; Rayan, A. 2012. Histopathological changes in some organs of males rats fed on genetically modified corn (Ajeeb YG). *Journal of American Science*, 8(10).

Ajeeb YG is a genetically modified (GM) insect resistant corn produced by incorporated the MON 810 (Monsanto) borer resistance trait in the best corn germplasm Ajeeb. The safety of Ajeeb YG corn was assessed by comparison of toxicology response variables in rats consuming diets containing Ajeeb YG with those containing Ajeeb corn grains. Corn grains from Ajeeb YG or Ajeeb were incorborated into rodent diets at 30% concentrations administered to rats (n= 10/group) for 91 days. An additional negative control group of rats (n= 10/group) were fed AIN93G diets. Rats fed on GM corn showed histopathological changes. Liver displayed cytoplasmic vacuolation of centrolobular hepatocytes and fatty degeneration of hepatocytes. Kidneys showed congestion of renal blood vessels and cystic dilatation of renal tubules. Testes revealed necrosis and desquamation of spermatogoneal germ cells lining seminiferous tubules. Spleen showed slight lymphocytic depletion and splenic congestion. Small intestine showed hyperplasia, hyperactivation of mucous secretory glands and necrosis of intestinal villi were detected. Due to these observations, we suggest that the risk of GM crops cannot be ignored and deserves further investigations in order to identify possible long-term effects, if any, of GM food consumption that might help in the post market surveillance.

Artigo completo disponível em http://www.jofamericanscience.org/journals/am-sci/am0810/093_11743am0810_684_696.pdf

Carman, J.; Vlieger, H.; Ver Steeg, L.; Sneller, V.; Robinson, G.; Clinch-Jones, C.; Haynes, J.; Edwards, J. 2013. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. *Journal of Organic Systems*, 8 (1): 38-54[55].

A significant number of genetically modified (GM) crops have been approved to enter human food and animal feed since 1996, including crops containing several GM genes 'stacked' into the one plant. We randomised and fed isowean pigs (N=168) either a mixed GM soy and GM corn (maize) diet (N=84) or an equivalent non-GM diet (N=84) in a longterm toxicology study of 22.7 weeks (the normal lifespan of a commercial pig from weaning to slaughter). Equal numbers of male and female pigs were present in each group. The GM corn contained double and triple-stacked varieties. Feed intake, weight gain, mortality and blood biochemistry were measured. Organ weights and pathology were determined post-mortem. There were no differences between pigs fed the GM and non-GM diets for feed intake, weight gain, mortality, and routine blood biochemistry measurements. The GM diet was associated with gastric and uterine differences in pigs. GM-fed pigs had uteri that were 25% heavier than non-GM fed pigs (p=0.025). GM-fed pigs had a higher rate of severe stomach inflammation with a rate of 32% of GM-fed pigs compared to 12% of non-GM-fed pigs (p=0.004). The severe stomach inflammation was worse in GM-fed males compared to non-GM fed males by a factor of 4.0 (p=0.041), and GM-fed females compared to non-GM fed females by a factor of 2.2 (p=0.034).

Artigo completo disponível em http://www.organic-systems.org/journal/81/8106.pdf

2 Riscos para a saúde associados ao uso de plantas Tolerantes a Herbicidas

2.1 Impactos negativos específicos relacionados aos principais herbicidas associados ao cultivo de plantas transgênicas do tipo TH

Grande parte dos riscos para a saúde humana, envolvendo o plantio, o consumo ou a manipulação de plantas transgênicas do tipo TH, provém da relação intrínseca que elas mantêm com determinados herbicidas.

Não obstante esse fato, as avaliações de risco para a saúde (e para o meio ambiente) validadas no Brasil pela Comissão Técnica Nacional de Biossegurança (CTNBio) desconsideram os herbicidas, não avaliam a presença de seus resíduos tóxicos nas lavouras e nos alimentos transgênicos.

Os artigos reunidos a seguir exemplificam alguns tipos de danos à

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[55] Esse estudo usou como base da dieta Geneticamente Modificada uma mistura de milho tolerante ao glifosato, milho Bt e soja tolerante ao glifosato. Por isto, essa referência consta em três itens dessa parte, sendo 1.4, 2.2.1 e 2.2.2.

saúde humana, provenientes do contato com agrotóxicos sistematicamente associados ao cultivo das plantas TH (formulações comerciais de herbicidas à base de glifosato, 2,4-D e glufosinato de amônio, em especial). O foco, nesse item, se centra na saúde do trabalhador rural.

Hardell, L.; Eriksson, M.; Lenner, P.; Lundgren, E. 1981. Malignant lymphoma and exposure to chemicals especially organic solvents, chlorophenols and phenoxy acids: A case-control study. *Br J Cancer*, 43:169-176.

A number of men with malignant lymphoma of the histiocytic type and previous exposure to phenoxy acids or chlorophenols were observed and reported in 1979. A matched case-control study has therefore been performed with cases of malignant lymphoma (Hodgkin's disease and non-Hodgkin lymphoma). This study included 169 cases and 338 controls. The results indicate that exposure to phenoxy acids, chlorophenols, and organic solvents may be a causative factor in malignant lymphoma. Combined exposure of these chemicals seemed to increase the risk. Exposure to various other agents was not obviously different in cases and in controls.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2010513/

Hietanen, E.; Linnainmaa, K.; Vainio, H. 1983. Effects of phenoxy herbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. *Acta Pharmacol Toxicol*, 53, 103-112.

The effects of phenoxyacid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), clofibrate, and glyphosate on hepatic and intestinal drug metabolizing enzyme activities were studied in rats intragastrically exposed for 2 weeks. The hepatic ethoxycoumarin O-deethylase activity increased about 2-fold with MCPA. Both 2,4-D and MCPA increased the hepatic epoxide hydrolase activity and decreased the hepatic glutathione S-transferase activity. MCPA also increased the intestinal activities of ethoxycoumarin O-deethylase and epoxide hydrolase. Glyphosate decreased the hepatic level of cytochrome P-450 and monooxygenase activities and the intestinal activity of aryl hydrocarbon hydroxylase. Clofibrate decreased the hepatic activities of UDPglucuronosyltransferase with p-nitrophenol or methylumbelliferone as the substrate. Also 2,4-D decreased the hepatic activity of UDPglucuronosyltransferase with p-nitrophenol as the substrate. MCPA decreased the intestinal activities of UDPglucuronosyltransferase with either p-nitrophenol or methylumbelliferone as the substrate. The results indicate that phenoxyacetic acids, especially MCPA, may have potent effects on the metabolism of xenobiotics. Glyphosate, not chemically related to phenoxyacids, seems to inhibit monooxygenases. Whether these changes are related to the toxicity of these xenobiotics remains to be clarified in further experiments.

http://www.ncbi.nlm.nih.gov/pubmed/6624478

EPI - Savitz, D.; Arbuckle, T.; Kaczor, D.; Curtis, K. 1997. Male pesticide exposure and pregnancy outcome. *Am J Epidemiol*, 146(12): 1025-1036.

Potential health effects of agricultural pesticide use include reproductive outcomes. For the Ontario Farm Family Health Study, the authors sampled Ontario farms from the 1986 Canadian Census of Agriculture, identified farm couples, and obtained questionnaire data concerning farm activities, reproductive health experience, and chemical applications. Male farm activities in the period from 3 months before conception through the month of conception were evaluated in relation to miscarriage, preterm delivery, and small-for-gestational-age births. Among the 1,898 couples with complete data (64% response), 3,984 eligible pregnancies were identified. Miscarriage was not associated with chemical activities overall but was increased in combination with reported use of thiocarbamates, carbaryl, and unclassified pesticides on the farm. Preterm delivery was also not strongly associated with farm chemical activities overall, except for mixing or applying yard herbicides (odds ratio = 2.1, 95% confidence interval 1.0-4.4). Combinations of activities with a variety of chemicals (atrazine, glyphosate, organophosphates, 4-[2,4-dichlorophenoxy] butyric acid, and insecticides) generated odds ratios of two or greater. No associations were found between farm chemicals and small-for-gestational-age births or altered sex ratio. Based on these data, despite limitations in exposure assessment, the authors encourage continued evaluation of male exposures, particularly in relation to miscarriage and preterm delivery.

http://www.ncbi.nlm.nih.gov/pubmed/9420527

EPI - McDuffie, H.; Pahwa, P.; McLaughlin, J.; Spinelli, J.; Fincham, S.; Dosman, J.; Robson, D.; Skinnider, L.; Choi, N. 2001. Non-Hodgkin's lymphoma and specific pesticide exposures in men: Cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev*, 10(11):1155-63.

Our objective in the study was to investigate the putative associations of specific pesticides with non-Hodgkin's Lymphoma [NHL; International Classification of Diseases, version 9 (ICD-9) 200, 202]. We conducted a Canadian multicenter population-based incident, case (n = 517)-control (n = 1506) study among men in a diversity of occupations using an initial postal questionnaire followed by a telephone interview for those reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder. Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the matching variables of age and province of residence, and subsequently adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization treatment, and a positive history of cancer in first-degree relatives). We found that among major chemical classes of herbicides, the risk of NHL was statistically significantly increased by exposure to phenoxyherbicides [OR, 1.38; 95% confidence interval (CI), 1.06-1.81] and to dicamba (OR, 1.88; 95% CI, 1.32-2.68). Exposure to carbamate (OR, 1.92; 95% CI, 1.22-3.04) and to organophosphorus insecticides (OR, 1.73; 95% CI, 1.27-2.36), amide fungicides, and the fumigant carbon tetrachloride (OR, 2.42; 95% CI, 1.19-5.14) statistically significantly increased risk. Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CI, 1.01-1.73), mecoprop (OR, 2.33; 95% CI, 1.58-3.44), and dicamba (OR, 1.68; 95% CI, 1.00-2.81); to the insecticides malathion (OR, 1.83; 95% CI, 1.31-2.55), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), carbaryl (OR, 2.11; 95% CI, 1.21-3.69), aldrin, and lindane; and to the fungicides captan and sulfur compounds. In additional multivariate models, which included exposure to other major chemical classes or individual pesticides, personal antecedent cancer, a history of cancer among first-degree relatives, and exposure to mixtures containing dicamba (OR, 1.96; 95% CI, 1.40-2.75) or to mecoprop (OR, 2.22; 95% CI, 1.49-3.29) and to aldrin (OR, 3.42; 95% CI, 1.18-9.95) were significant independent predictors of an increased risk for NHL, whereas a personal history of measles and of allergy desensitization treatments lowered the risk. We concluded that NHL was associated with specific pesticides after adjustment for other independent predictors.

http://www.ncbi.nlm.nih.gov/pubmed/11700263

EPI - Garry, V.; Harkins, M.; Erickson, L.; Long-Simpson, L.; Holland, S.; Burroughs, B. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the red river Valley of Minnesota, USA. *Environmental Health Perspectives*, Volume 110, Supplement 3.

We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989-1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children, conducted during 1997-1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent-reported birth defects. In the present study, in the first year of life, the birth defect rate was 31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2-5.1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3-9.6) in the neurobehavioral category. Finally, these studies point out that (a) herbicides applied in the spring may be a factor in the birth defects observed and (b) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241196/pdf/ehp110s-000441.pdf

EPI – Hardell, L.; Eriksson, M.; Nordstrom, M. 2002. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma*, 43(5): 1043-1049.

Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in univariate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26-2.42), insecticides (OR 1.43, CI 95% 1.08-1.87), fungicides (OR 3.11, CI 95% 1.56-6.27) and impregnating agents (OR 1.48, CI 95% 1.11-1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95% 1.08-8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40-4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before diagnosis. However, in multivariate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.

http://www.ncbi.nlm.nih.gov/pubmed/12148884

Greenlee, A.; Ellis, T.; Berg, R. 2004. Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos. *Environmental Health Perspectives*, vol. 112 (6), p. 703-709.

Occupational exposures to pesticides may increase parental risk of infertility and adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, and congenital anomalies. Less is known about residential use of pesticides and the risks they pose to reproduction and development. In the present study we evaluate environmentally relevant, low-dose exposures to agrochemicals and lawn-care pesticides for their direct effects on mouse preimplantation embryo development, a period corresponding to the first 5-7 days after human conception. Agents tested were those commonly used in the upper midwestern United States, including six herbicides [atrazine, dicamba, metolachlor, 2,4-dichlorophenoxyacetic acid (2,4-D)], pendimethalin, and mecoprop), three insecticides (chlorpyrifos, terbufos, and permethrin), two fungicides (chlorothalonil and mancozeb), a desiccant (diquat), and a fertilizer (ammonium nitrate). Groups of 20-25 embryos were incubated 96 hr in vitro with either individual chemicals or mixtures of chemicals simulating exposures encountered by handling pesticides, inhaling drift, or ingesting contaminated groundwater. Incubating embryos with individual pesticides increased the percentage of apoptosis (cell death) for 11 of 13 chemicals (p <or= 0.05) and reduced development to blastocyst and mean cell number per embryo for 3 of 13 agents (p <or= 0.05). Mixtures simulating preemergent herbicides, postemergent herbicides, and fungicides increased the percentage of apoptosis in exposed embryos (p <or= 0.05). Mixtures simulating groundwater contaminants, insecticide formulation, and lawn-care herbicides reduced development to blastocyst and mean cell number per embryo (p <or= 0.05). Our data demonstrate that pesticide-induced injury can occur very early in development, with a variety of agents, and at concentrations assumed to be without adverse health consequences for humans.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241965/pdf/ehp0112-000703.pdf

REV – Kamel, F.; Hoppin, J. 2004. Association of pesticide exposure with neurologic dysfunction and disease. *Environmental Health Perspectives*, Volume 112, number 9.

Poisoning by acute high-level exposure to certain pesticides has well-known neurotoxic effects, but whether chronic exposure to moderate levels of pesticides is also neurotoxic is more controversial. Most studies of moderate pesticide exposure have found increased prevalence of neurologic symptoms and changes in neurobehavioral performance, reflecting cognitive and psychomotor dysfunction. There is less evidence that moderate exposure is related to deficits in sensory or motor function or peripheral nerve conduction, but fewer studies have considered these outcomes. It is possible that the most sensitive manifestation of pesticide neurotoxicity is a general malaise lacking in specificity and related to mild cognitive dysfunction, similar to that described for Gulf War syndrome. Most studies have focused on organophosphate insecticides, but some found neuro-toxic effects from other pesticides, including fungicides, fumigants, and organochlorine and carbamate insecticides. Pesticide exposure may also be associated with increased risk of Parkinson disease; several classes of pesticides, including insecticides, herbicides, and fungicides, have been implicated. Studies of other neurodegenerative diseases are limited and inconclusive. Future studies will need to improve assessment of pesticide exposure in individuals and consider the role of genetic susceptibility. More studies of pesticides other than organophosphates are needed. Major unresolved issues include the relative importance of acute and chronic exposure, the effect of moderate exposure in the absence of poisoning, and the relationship of pesticide-related neurotoxicity to neurodegenerative disease.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1247187/

EPI – Rull, R.; Ritz, B.; Shaw, G. 2006. Neural tube defects and maternal residential proximity to agricultural pesticide applications. *American Journal of Epidemiology*, 163 (8): 743-753.

Residential proximity to applications of agricultural pesticides may be an important source of exposure to agents that have been classified as developmental toxins. Data on two case-control study populations of infants with neural tube defects (NTDs) and nonmalformed controls delivered in California between 1987 and 1991 were pooled to investigate whether maternal residential proximity to applications of specific pesticides or physicochemical groups of pesticides during early gestation increases the risk of these malformations. Maternal residential proximity within 1,000 m of pesticide applications was ascertained by linking mothers' addresses with agricultural pesticide use reports and crop maps. Odds ratios were computed by using conventional single- and multiple-pesticide and hierarchical multiple-pesticide logistic regression. In single-pesticide models, several pesticides were associated with NTDs after adjustment for study population, maternal ethnicity, educational level, cigarette smoking, and vitamin use. In a hierarchical multiple-pesticide model, effect estimates for only benomyl and methomyl suggested a possible association. Elevated risks of NTDs and anencephaly or spina bifida subtypes were also associated with exposures to chemicals classified as amide, benzimidazole, methyl carbamate, or organophosphorus pesticides and with increasing numbers of pesticides. These results suggest that ambient exposure to certain categories of agricultural pesticides may increase the risk of NTDs.

Artigo completo disponível em http://aje.oxfordjournals.org/content/163/8/743.long

REV - Benitez-Leite, S.; Macchi, M.; Acosta, M. 2007. Malformaciones congénitas asociadas a agrotóxicos. *Pediatr (Asunción)*, Vol. 34; nº 2; 2007.

Introducción: La exposición a plaguicidas es un riesgo reconocido para la salud humana. Se describe la relación entre la exposición de los padres y malformaciones congénitas en el neonato.
Objetivo: Estudiar la asociación entre la exposición a pesticidas y malformaciones congénitas en neonatos nacidos en el Hospital Regional de Encarnación, Itapúa- Paraguay.
Material y Método: Estudio prospectivo de casos y controles de marzo/2006 a febrero/2007. Se consideró caso a todo neonato con malformación congénita, y control a todo niño sano del mismo sexo que naciera inmediatamente después. No se incluyeron los nacimientos ocurridos fuera del hospital. Se consideró exposición a cualquier contacto con agroquímicos, así como a otros factores de riesgo conocidos para malformación congénita.
Resultados: Se analizaron 52 casos y 87 controles. El promedio de nacimientos por mes fue de 216. Los factores de riesgo asociados significativamente fueron: vivir cerca de campos fumigados (OR 2.46 IC95%1.09- 5.57,p<0,02), vivienda ubicada a <1Km (OR=2,66 IC 95% 1,19 – 5,97), p<0,008), almacenamiento de plaguicidas en el hogar (OR 15,35 IC95%1.96-701,63 p<0.003), contacto en forma directa o accidental con plaguicidas(OR3.19 IC95%0,97-11.4, p<0.04), antecedente de malformación en la familia (OR 6,81 IC5% 1.94-30,56, p<0.001). Los demás factores de riesgo conocidos para malformaciones no tuvieron significancia estadística.
Conclusión: Los resultados muestran una asociación entre exposición a pesticidas y malformaciones congénitas. Se requiere de estudios futuros para confirmar estos hallazgos.

Artigo completo disponível em http://scielo.iics.una.py/scielo.php?script=sci_arttext&pid=S1683-98032007000200002&lng=pt&nrm=iso

REV - Eriksson, M.; Hardell, L.; Carlberg, M.; Akerman, M. 2008. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *International Journal of Cancer*, 123,1657-1663.

We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91 %) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for

MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with >10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.

http://www.ncbi.nlm.nih.gov/pubmed/18623080

Benedetti, D.; Nunes, E.; Sarmento, M.; Porto, C.; Santos, C.; Dias, J.; Silva, J. 2013. Genetic damage in soybean workers exposed to pesticides: evaluation with the comet and buccal micronucleus cytome assays. *Mutat Res*, 752(1-2): 28-33.

Soybean cultivation is widespread in the State of Rio Grande do Sul (RS, Brazil), especially in the city of Espumoso. Soybean workers in this region are increasingly exposed to a wide combination of chemical agents present in formulations of fungicides, herbicides, and insecticides. In the present study, the comet assay in peripheral leukocytes and the buccal micronucleus (MN) cytome assay (BMCyt) in exfoliated buccal cells were used to assess the effects of exposures to pesticides in soybean farm workers from Espumoso. A total of 127 individuals, 81 exposed and 46 non-exposed controls, were evaluated. Comet assay and BMCyt (micronuclei and nuclear buds) data revealed DNA damage in soybean workers. Cell death was also observed (condensed chromatin, karyorhectic, and karyolitic cells). Inhibition of non-specific choline esterase (BchE) was not observed in the workers. The trace element contents of buccal samples were analyzed by Particle-Induced X-ray Emission (PIXE). Higher concentrations of Mg, Al, Si, P, S, and Cl were observed in cells from workers. No associations with use of personal protective equipment, gender, or mode of application of pesticides were observed. Our findings indicate the advisability of monitoring genetic toxicity in soybean farm workers exposed to pesticides.

http://www.ncbi.nlm.nih.gov/pubmed/23347873

Jayasumana, C.; Gunatilake, S.; Senanayake, P. 2014. Glyphosate, hard water and nephrotoxic metals: Are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? *Int J Environ Res Public Health*, *11*, 2125-2147; doi:10.3390/ijerph110202125.

The current chronic kidney disease epidemic, the major health issue in the rice paddy farming areas in Sri Lanka has been the subject of many scientific and political debates over the last decade. Although there is no agreement among scientists about the etiology of the disease, a majority of them has concluded that this is a toxic nephropathy. None of the hypotheses put forward so far could explain coherently the totality of clinical, biochemical, histopathological findings, and the unique geographical distribution of the disease and its appearance in the mid-1990s. A strong association between the consumption of hard water and the occurrence of this special kidney disease has been observed, but the relationship has not been explained consistently. Here, we have hypothesized the association of using glyphosate, the most widely used herbicide in the disease endemic area and its unique metal chelating properties. The possible role played by glyphosate-metal complexes in this epidemic has not been given any serious consideration by investigators for the last two decades. Furthermore, it may explain similar kidney disease epidemics observed in Andra Pradesh (India) and Central America. Although glyphosate alone does not cause an epidemic of chronic kidney disease, it seems to have acquired the ability to destroy the renal tissues of thousands of farmers when it forms complexes with a localized geo environmental factor (hardness) and nephrotoxic metals.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3945589/

Enquanto os trabalhadores rurais se fazem ameaçados por exposição direta, quando a absorção dos venenos por se dar também por contato e inalação e as intoxicações agudas tendem a ser claramente percebidas, no caso dos consumidores urbanos os problemas tendem a ser crônicos. O contato com subdosagens, incorporadas ao consumo diário de alimentos contaminados por resíduos de herbicidas, tende a ser de difícil percepção. As relações de causa e efeito, nessas circunstâncias, se mostram tênues e difusas, exigindo protocolos de análise bastante distintos daqueles adotados em avaliações expeditas.

Os estudos a seguir mostram que, por questões de metabolismo decorrente da transformação genética, os herbicidas associados ao cultivo de plantas GM TH tendem a se acumular em níveis maiores nas células das plantas transgênicas do que nas plantas convencionais. As implicações em termos de riscos para o consumo humano e animal decorrem dos maiores níveis de exposição aos herbicidas, quando a alimentação inclui partes daquelas PGMs.

Duke, S.; Rimando, A.; Pace, P.; Reddy, K.; Smeda, R. 2003. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. *J Agric Food Chem*, *51,* 340-344.

The estrogenic isoflavones of soybeans and their glycosides are products of the shikimate pathway, the target pathway of glyphosate. This study tested the hypothesis that nonphytotoxic levels of glyphosate and other herbicides known to affect phenolic compound biosynthesis might influence levels of these nutraceutical compounds in glyphosate-resistant soybeans. The effects of glyphosate and other herbicides were determined on estrogenic isoflavones and shikimate in glyphosate-resistant soybeans from identical experiments conducted on different cultivars in Mississippi and Missouri. Four commonly used herbicide treatments were compared to a hand-weeded control. The herbicide treatments were (1) glyphosate at 1260 g/ha at 3 weeks after planting (WAP), followed by glyphosate at 840 g/ha at 6 WAP; (2) sulfentrazone at 168 g/ha plus chlorimuron at 34 g/ha applied preemergence (PRE), followed by glyphosate at 1260 g/ha at 6 WAP; (3) sulfentrazone at 168 g/ha plus chlorimuron at 34 g/ha applied PRE, followed by glyphosate at 1260 g/ha at full bloom; and (4) sulfentrazone at 168 g/ha plus chlorimuron at 34 g/ha applied PRE, followed by acifluorfen at 280 g/ha plus bentazon at 560 g/ha plus clethodim at 140 g/ha at 6 WAP. Soybeans were harvested at maturity, and seeds were analyzed for daidzein, daidzin, genistein, genistin, glycitin, glycitein, shikimate, glyphosate, and the glyphosate degradation product, aminomethylphosphonic acid (AMPA). There were no remarkable effects of any treatment on the contents of any of the biosynthetic compounds in soybean seed from either test site, indicating that early and later season applications of glyphosate have no effects on phytoestrogen levels in glyphosate-resistant soybeans. Glyphosate and AMPA residues were higher in seeds from treatment 3 than from the other two treatments in which glyphosate was used earlier. Intermediate levels were found in treatments 1 and 2. Low levels of glyphosate and AMPA were found in treatment 4 and a hand-weeded control, apparently due to herbicide drift.

http://www.ncbi.nlm.nih.gov/pubmed/12502430

Arregui, M.; Lenardón, A.; Sanchez, D.; Maitre, M.; Scotta, R.; Enrique, S. 2004. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. *Pest Manag Sci*, 60:163-166.

The availability of Roundup Ready (RR) varieties of soybean has increased the use of glyphosate for weed control in Argentina. Glyphosate [(N-phosphonomethyl)glycine] is employed for the eradication of previous crop vegetation and for weed control during the soybean growing cycle. Its action is effective, and low environmental impact has been reported so far. No residues have been observed in soil or water, either of glyphosate or its metabolite, AMPA (aminomethylphosphonic acid). The objective of this work was to monitor glyphosate and AMPA residues in soybean plants and grains in field crops in Santa Fe Province, Argentina. Five sites were monitored in 1997, 1998 and 1999. Individual soybean plants were sampled from emergence to harvest, dried and ground. Analysis consisted in residue extraction with organic solvents and buffers, agitation, centrifugation, clean-up and HPLC with UV detection. In soybean leaves and stems, glyphosate residues ranged from 1.9 to 4.4 mg kg(-1) and from 0.1 to 1.8 mg kg(-1) in grains. Higher concentrations were detected when glyphosate was sprayed several times during the crop cycle, and when treatments approached the flowering stage. AMPA residues were also detected in leaves and in grains, indicating metabolism of the herbicide.

http://www.ncbi.nlm.nih.gov/pubmed/14971683

Bøhn, T.; Cuhra, M.; Traavik, T.; Sanden, M.; Fagan, J.; Primicerio, R. 2014. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. *Food Chemistry*, 153, 207–215.

This article describes the nutrient and elemental composition, including residues of herbicides and pesticides, of 31 soybean batches from Iowa, USA. The soy samples were grouped into three different categories: (i) genetically modified, glyphosate-tolerant soy (GM-soy); (ii) unmodified soy cultivated using a conventional "chemical" cultivation regime; and (iii) unmodified soy cultivated using an organic cultivation regime. Organic soybeans showed the healthiest nutritional profile with more sugars, such as glucose, fructose, sucrose and maltose, significantly more total protein, zinc and less fibre than both conventional and GM-soy. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soy. GM-soy contained high residues of glyphosate and AMPA (mean 3.3 and 5.7mg/kg, respectively). Conventional and organic soybean batches contained none of these agrochemicals. Using 35 different nutritional and elemental variables to characterise each soy sample, we were able to discriminate GM, conventional and organic soybeans without exception, demonstrating "substantial non-equivalence" in compositional characteristics for 'ready-to-market' soybeans.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0308814613019201

Ainda em 2011 se percebem deficiências de informações consistentes, que suportem avaliação de riscos em PGMs TH. É escassa, quase nula, a disponibilidade de estudos e dados sistematizados sobre graus de absorção e acúmulo de herbicidas em plantas GM TH e suas partes destinadas ao consumo.

Kleter, G.; Unsworth, J.; Harris, C. 2011. The impact of altered herbicide residues in transgenic herbicide-resistant crops on standard setting for herbicide residues. *Pest Management Science*, 67, 10: 1193–1210. DOI 10.1002/ps.2128.

The global area covered with transgenic (genetically modified) crops has rapidly increased since their introduction in the mid-1990s. Most of these crops have been rendered herbicide resistant, for which it can be envisaged that the modification has an impact on the profile and level of herbicide residues within these crops. In this article, the four main categories of herbicide resistance, including resistance to acetolactate-synthase inhibitors, bromoxynil, glufosinate and glyphosate, are reviewed. The topics considered are the molecular mechanism underlying the herbicide resistance, the nature and levels of the residues formed and their impact on the residue definition and maximum residue limits (MRLs) defined by the Codex Alimentarius Commission and national authorities. No general conclusions can be drawn concerning the nature and level of residues, which has to be done on a case-by-case basis. International residue definitions and MRLs are still lacking for some herbicide-crop combinations, and harmonisation is therefore recommended.

http://www.ncbi.nlm.nih.gov/pubmed/21898904

Avaliações de riscos para a saúde, por ocasião do consumo de plantas TH, devem levar em conta elementos qualitativos e quantitativos. Sabe-se que determinados herbicidas apresentam um metabolismo de degradação diferente, em plantas GM, comparativamente ao que ocorre em plantas isogênicas não GM de onde as outras se originam. Isso determina que nas diferentes plantas haverão produtos tóxicos distintos, decorrentes do contato com aqueles herbicidas. Naturalmente isto implicará em riscos distintos, que se ampliarão em função dos aspectos quantitativos relacionados à presença – nas PGMs – de maiores volumes de resíduos (comparativamente ao que ocorre nas lavouras tradicionais).

Droge-Laser, W.; Siemeling, U.; Pühler, A.; Broer, I. 1994. The metabolites of the herbicide L-Phosphinothricin (GIufosinate) (Identification, stability, and mobility in transgenic, herbicide-resistant, and untransformed plants). *Plant Physiology*, 105: 159-166.

The metabolism of the herbicide L-phosphinothricin (L-Pt) was analyzed in tobacco (Nicotiana tabacum), alfalfa (Medicago sativa), and carrot (Daucus carota). In transgenic, Pt-resistant plants expressing the Pt-N-acetyltransferase gene (pat), L-Pt was acetylated, resulting in two forms of N-acetyl-Pt (ac-Pt). In transgenic plants expressing only low pat-encoded acetylating activity as well as in genetically unmodified plants, three metabolic compounds 4-methylphosphinico-2-oxo-butanoic acid, 3-methylphosphinico-propanoic acid (MPP), and 4-methylphosphinico-2-hydroxy-butanoic acid (MHB) were identified. Hence, the transgene-encoded acetylation of L-Pt competes with a plant-specific degradation. The compounds MPP, MHB, and ac-Pt were found to be the final, stable products of the plant's metabolic pathways. The mobility of these stable compounds in the plant was investigated: L-Pt as well as the derived metabolites were found to be preferentially transported to the upper regions of the plant.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC159341/

Laurent, F.; Debrauwer, L.; Rathahao, E.; Scalla, R. 2000. 2,4-Dichlorophenoxyacetic acid metabolism in transgenic tolerant cotton (*Gossypium hirsutum*). *J Agric Food Chem*, 48, 307–5311.

The metabolic fate of 2,4-dichlorophenoxyacetic acid (2,4-D) was studied in leaves of transgenic 2,4-D-tolerant cotton (Gossypium hirsutum), which is obtained by transfer of the tfdA gene from the bacterium Alcaligenes eutrophus. The tdfA gene codes for a dioxygenase catalyzing the degradation of 2,4-D to 2,4-dichlorophenol (2,4-DCP). [phenyl-(14)C]-2,4-D was administered by petiolar absorption followed by an 18 h water chase or converted to the isopropyl ester and sprayed onto the leaf surface; the leaves were harvested 48 h later. The herbicide was degraded to 2,4-DCP by the bacterial enzyme expressed in the plants. 2,4-DCP was rapidly converted to more polar metabolites and was never found in detectable amounts. Metabolite structures were deduced from enzymatic hydrolysis studies and mass spectrometric analyses. The first metabolite was the glucoside conjugate of 2,4-DCP (2, 4-DCP-beta-O-glucoside). The major terminal metabolites were two more complex glucosides: 2,4-DCP-(6-O-malonyl)glucoside and 2, 4-DCP-(6-O-sulfate) glucoside.

http://www.ncbi.nlm.nih.gov/pubmed/11087477

Müller, B.; Zumdick, A.; Schuphan, I.; Schmidt, B. 2001. Metabolism of the herbicide glufosinate-ammonium in plant cell cultures of transgenic (rhizomania-resistant) and non-transgenic sugarbeet (*Beta vulgaris*), carrot (*Daucus carota*), Purple foxglove (*Digitalis purpurea*) and thorn apple (*Datura stramonium*). *Pest Manag Sci*, 57, 46-56.

The metabolism of the herbicide glufosinate-ammonium was investigated in heterotrophic cell suspension and callus cultures of transgenic (bar-gene) and non-transgenic sugarbeet (Beta vulgaris). Similar studies were performed with suspensions of carrot (Daucus carota), purple foxglove (Digitalis purpurea) and thorn apple (Datura stramonium). 14C-labelled chemicals were the (racemic) glufosinate, L-glufosinate, and D-glufosinate, as well as the metabolites N-acetyl L-glufosinate and 3-(hydroxymethylphosphinyl)propionic acid (MPP). Cellular absorption was generally low, but depended noticeably on plant species, substance and enantiomer. Portions of non-extractable residues ranged from 0.1% to 1.2% of applied 14C. Amounts of soluble metabolites resulting from glufosinate or L-glufosinate were between 0.0% and 26.7% of absorbed 14C in non-transgenic cultures and 28.2% and 59.9% in transgenic sugarbeet. D-Glufosinate, MPP and N-acetyl L-glufosinate proved to be stable. The main metabolite in transgenic sugarbeet was N-acetyl L-glufosinate, besides traces of MPP and 4-(hydroxymethylphosphinyl)butanoic acid (MPB). In non-transgenic sugarbeet, glufosinate was transformed to a limited extent to MPP and trace amounts of MPB. In carrot, D stramonium and D purpurea, MPP was also the main product; MPB was identified as a further trace metabolite in D stramonium and D purpurea.

http://www.ncbi.nlm.nih.gov/pubmed/11455632

Laurent, F.; Debrauwer, L.; Pascal-Lorber, S. 2006. Metabolism of [14C]-2,4-dichlorophenol in edible plants. *Pest Manag Sci*, 62, 558–564.

Several 2,4-dichlorophenoxyacetic acid (2,4-D)-sensitive plants have been modified by genetic engineering with tfdA gene to acquire 2,4-D tolerance. The expression product of this gene degrades 2,4-D to 2,4-dichlorophenol (DCP), which is less phytotoxic but could cause a problem of food safety. After a comparison of 2,4-D and DCP metabolism in transgenic 2,4-D-tolerant and wild cotton (Gossypium hirsutum L.), a direct study of DCP metabolism in edible plants was performed. After petiolar uptake of a [U-phenyl-(14)C]-DCP solution followed by a 48 h water chase, aqueous extracts were analysed by high-performance liquid chromatography. Metabolites were thereafter isolated and their structural identities were determined by enzymatic and chemical hydrolyses and mass spectrometry analyses. The metabolic fate of DCP was equivalent to 2,4-D metabolism in transgenic 2,4-D-tolerant cotton. In addition, DCP metabolism was similar in transgenic and wild cotton. The major terminal metabolites were DCP-saccharide conjugates in all species, essentially DCP-(6-O-malonyl)-glucoside or its precursor DCP-glucose. The significance of this metabolic pathway with regard to food safety is discussed.

http://www.ncbi.nlm.nih.gov/pubmed/16628540

2.1.1 O princípio ativo glifosato e seu principal metabólito de degradação (o ácido aminometilfosfônico – AMPA)

Apresentado como de baixa toxidade e baixa persistência no ambiente, o glifosato tem sido beneficiado por uma imagem pública relativamente positiva, que se estende ao Roundup, formulação comercial responsável pelo maior volume de vendas daquele princípio ativo. Entretanto, estudos recentes contrariam aquela imagem, associando sua presença a reações citotóxicas e cancerígenas, bem como a impactos negativos sobre a flora digestiva. O ácido aminometilfosfônico (AMPA), principal metabólito de degradação do glifosato, também oferece propriedades tóxicas e genotóxicas.

Hietanen, E.; Linnainmaa, K.; Vainio, H. 1983. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. *Acta Pharmacologica et Toxicologica (Copenh)*, 53(2):103-12.

The effects of phenoxyacid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), clofibrate, and glyphosate on hepatic and intestinal drug metabolizing enzyme activities were studied in rats intragastrically exposed for 2 weeks. The hepatic ethoxycoumarin O-deethylase activity increased about 2-fold with MCPA. Both 2,4-D and MCPA increased the hepatic epoxide hydrolase activity and decreased the hepatic glutathione S-transferase activity. MCPA also increased the intestinal activities of ethoxycoumarin O-deethylase and epoxide hydrolase. Glyphosate decreased the hepatic level of cytochrome P-450 and monooxygenase activities and the intestinal activity of aryl hydrocarbon hydroxylase. Clofibrate decreased the hepatic activities of UDPglucuronosyltransferase with p-nitrophenol or methylumbelliferone as the substrate. Also 2,4-D decreased the hepatic activity of UDPglucuronosyltransferase with p-nitrophenol as the substrate. MCPA decreased the intestinal activities of UDPglucuronosyltransferase with either p-nitrophenol or methylumbelliferone as the substrate. The results indicate that phenoxyacetic acids, especially MCPA, may have potent effects on the metabolism of xenobiotics. Glyphosate, not chemically related to phenoxyacids, seems to inhibit monooxygenases. Whether these changes are related to the toxicity of these xenobiotics remains to be clarified in further experiments.

http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0773.1983.tb01876.x/abstract

Lioi, M.; Scarf, M.; Santoro, A.; Barbieri, R.; Zeni, O.; Berardino, D.; Ursini, M. 1998. Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures *in vitro*. *Mutation Research*, 403, 13–20.

The genotoxic activity of the pesticides gliphosate, vinclozolin and DPX-E9636 was studied in in vitro cultures of bovine lymphocytes, using chromosome aberration (CA) and sister chromatid exchange (SCE) frequencies as genetic end-points and a variation of glucose 6-phosphate dehydrogenase (G6PD) enzyme activity as a marker of changes in the normal cell redox state. Results indicated a statistically significant increase of structural aberrations, sister chromatid exchanges and G6PD activity, suggesting that the pesticides tested induce either oxidative stress or a mutagenic effect in this species. The evaluation of both mitotic index and cell viability, after pesticide exposure, demonstrates a high cytotoxic effect which is always associated with the observed genotoxic effect.

http://www.ncbi.nlm.nih.gov/pubmed/9726001

Daruich, J.; Zirulnik, F.; Gimenez, M. 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses. *Environmental Research Section*, A 85**,** 226-231.

To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organophosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.

http://www.ncbi.nlm.nih.gov/pubmed/11237511

Beuret, C.; Zirulnik, F.; Giménez, M. 2005. Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. *Reproductive Toxicology*, 19, 501–504.

Glyphosate is a post-emergence herbicide that acts on the synthesis of amino acids and other endogenous metabolites in plants. It is commonly used in agriculture, forestry, and nurseries for the control or destruction of herbaceous plants. Metabolic processes during development and pregnancy could be sensitive to changes induced by glyphosate such as lipid peroxidation. The present study has investigated the effects that 1% glyphosate oral exposure has on lipoperoxidation and antioxidant enzyme systems in the maternal serum and liver of pregnant rats and their term fetuses at 21 days of gestation. The results suggest that excessive lipid peroxidation induced with glyphosate ingestion leads to an overload of maternal and fetal antioxidant defense systems.

http://www.ncbi.nlm.nih.gov/pubmed/15749264

Hokanson, R.; Fudge, R.; Chowdhary, R; Busbee, D. 2007. Alteration of estrogen regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. *Hum Exp Toxicol*, 26 (9): 747-52.

Gene expression is altered in mammalian cells (MCF-7 cells), by exposure to a variety of chemicals that mimic steroid hormones or interact with endocrine receptors or their co-factors. Among those populations chronically exposed to these endocrine disruptive chemicals are persons, and their families, who are employed in agriculture or horticulture, or who use agricultural/horticultural chemicals. Among the chemicals most commonly used, both commercially and in the home, is the herbicide glyphosate. Although glyphosate is commonly considered to be relatively non-toxic, we utilized in vitro DNA microarray analysis of this chemical to evaluate its capacity to alter the expression of a variety of genes in human cells. We selected a group of genes, determined by DNA microarray analysis to be dysregulated, and used quantitative real-time PCR to corroborate their altered states of expression. We discussed the reported function of those genes, with emphasis on altered physiological states that are capable of initiating adverse health effects that might be anticipated if gene expression were significantly altered in either adults or embryos exposed in utero.

http://www.ncbi.nlm.nih.gov/pubmed/17984146

Mañas, F.; Peralta, L.; Raviolo, J.; Ovando, H.; Weyers, A.; Ugnia, L.; Cid, M.; Larripa, I.; Gorla, N. 2009. Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicology and Environmental Safety*, 72.

Formulations containing glyphosate are the most widely used herbicides in the world. AMPA is the major environmental breakdown product of glyphosate. The purpose of this study is to evaluate the in vitro genotoxicity of AMPA using the Comet assay in Hep-2 cells after 4h of incubation and the chromosome aberration (CA) test in human lymphocytes after 48h of exposition. Potential in vivo genotoxicity was evaluated through the micronucleus test in mice. In the Comet assay, the level of DNA damage in exposed cells at 2.5-7.5mM showed a significant increase compared with the control group. In human lymphocytes we found statistically significant clastogenic effect AMPA at 1.8mM compared with the control group. In vivo, the micronucleus test rendered significant statistical increases at 200-400mg/kg. AMPA was genotoxic in the three performed tests. Very scarce data are available about AMPA potential genotoxicity.

http://www.ncbi.nlm.nih.gov/pubmed/19013644

Mañas, F.; Peralta, L.; Raviolo, J.; Ovando, H.; Weyers, A.; Ugnia, L.; Cid, M.; Larripa, I.; Gorla, N. 2009. Genotoxicity of glyphosate assessed by the Comet assay and cytogenic tests. *Environ Toxicol Pharmacol*, 28: 37–41.

It was evaluated the genotoxicity of glyphosate which up to now has heterogeneous results. The comet assay was performed in Hep-2 cells. The level of DNA damage in the control group (5.42±1.83 arbitrary units) for tail moment (TM) measurements has shown a significant increase ($p<0.01$) with glyphosate at a range concentration from 3.00 to 7.50mM. In the chromosome aberrations (CA) test in human lymphocytes the herbicide (0.20-6.00mM) showed no significant effects in comparison with the control group. In vivo, the micronucleus test (MNT) was evaluated in mice at three doses rendering statistical significant increases at 400mg/kg (13.0±3.08 micronucleated erythrocytes/1000 cells, $p<0.01$). In the present study glyphosate was genotoxic in the comet assay in Hep-2 cells and in the MNT test at 400mg/kg in mice. Thiobarbituric acid reactive substances (TBARs) levels, superoxide dismutase (SOD) and catalase (CAT) activities were quantified in their organs. The results showed an increase in these enzyme activities.

http://www.ncbi.nlm.nih.gov/pubmed/21783980

Thongprakaisang, S.; Thiantanawat, A.; Rangkadilok, N.; Suriyo, T.; Satayavivad, J. 2013. Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food and Chemical Toxicology*, doi: http://dx.doi.org/10.1016/j.fct.2013.05.057.

Glyphosate is an active ingredient of the most widely used herbicide and it is believed to be less toxic than other pesticides. However, several recent studies showed its potential adverse health effects to humans as it may be an endocrine disruptor. This study focuses on the effects of pure glyphosate on estrogen receptors (ERs) mediated transcriptional activity and their expressions. Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells, at 10(-12) to 10(-6)M in estrogen withdrawal condition. The proliferative concentrations of glyphosate that induced the activation of estrogen response element (ERE) transcription activity were 5-13 fold of control in T47D-KBluc cells and this activation was inhibited by an estrogen antagonist, ICI 182780, indicating that the estrogenic activity of glyphosate was mediated via ERs. Furthermore, glyphosate also altered both ERα and β expression. These results indicated that low and environmentally relevant concentrations of glyphosate possessed estrogenic activity. Glyphosate-based herbicides are widely used for soybean cultivation, and our results also found that there was an additive estrogenic effect between glyphosate and genistein, a phytoestrogen in soybeans. However, these additive effects of glyphosate contamination in soybeans need further animal study.

http://www.ncbi.nlm.nih.gov/pubmed/23756170

REV – Samsel, A.; Seneff, S. 2013. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. *Entropy*, 15: 1416–1463.

Glyphosate, the active ingredient in Roundup®, is the most popular herbicide used worldwide. The industry asserts it is minimally toxic to humans, but here we argue otherwise. Residues are found in the main foods of the Western diet, comprised primarily of sugar, corn, soy and wheat. Glyphosate's inhibition of cytochrome P450 (CYP) enzymes is an overlooked component of its toxicity to mammals. CYP enzymes play crucial roles in biology, one of which is to detoxify xenobiotics. Thus, glyphosate enhances the damaging effects of other food borne chemical residues and environmental toxins. Negative impact on the body is insidious and manifests slowly over time as inflammation damages cellular systems throughout the body. Here, we show how interference with CYP enzymes acts synergistically with disruption of the biosynthesis of aromatic amino acids by gut bacteria, as well as impairment in serum sulfate transport. Consequences are most of the diseases and conditions associated with a Western diet, which include gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's disease. We explain the documented effects of glyphosate and its ability to induce disease, and we show that glyphosate is the "textbook example" of exogenous semiotic entropy: the disruption of homeostasis by environmental toxins.

Artigo completo disponível em http://www.mdpi.com/1099-4300/15/4/1416

REV – Samsel, A.; Seneff, S. 2013. Glyphosate, pathways to modern diseases II: Celiac sprue and gluten intolerance. *Interdiscip Toxicol*, Vol. 6(4): 159–184. doi: 10.2478/intox-2013-0026.

Celiac disease, and, more generally, gluten intolerance, is a growing problem worldwide, but especially in North America and Europe, where an estimated 5% of the population now suffers from it. Symptoms include nausea, diarrhea, skin rashes, macrocytic anemia and depression. It is a multifactorial disease associated with numerous nutritional deficiencies as well as reproductive issues and increased risk to thyroid disease, kidney failure and cancer. Here, we propose that glyphosate, the active ingredient in the herbicide, Roundup®, is the most important causal factor in this epidemic. Fish exposed to glyphosate develop digestive problems that are reminiscent of celiac disease. Celiac disease is associated with imbalances in gut bacteria that can be fully explained by the known effects of glyphosate on gut bacteria. Characteristics of celiac disease point to impairment in many cytochrome P450 enzymes, which are involved with detoxifying environmental toxins, activating vitamin D3, catabolizing vitamin A, and maintaining bile acid production and sulfate supplies to the gut. Glyphosate is known to inhibit cytochrome P450 enzymes. Deficiencies in iron, cobalt, molybdenum, copper and other rare metals associated with celiac disease can be attributed to glyphosate's strong ability to chelate these elements. Deficiencies in tryptophan, tyrosine, methionine and selenomethionine associated with celiac disease match glyphosate's known depletion of these amino acids. Celiac disease patients have an increased risk to non-Hodgkin's lymphoma, which has also been implicated in glyphosate exposure. Reproductive issues associated with celiac disease, such as infertility, miscarriages, and birth defects, can also be explained by glyphosate. Glyphosate residues in wheat and other crops are likely increasing recently due to the growing practice of crop desiccation just prior to the harvest. We argue that the practice of "ripening" sugar cane with glyphosate may explain the recent surge in kidney failure among agricultural workers in Central America. We conclude with a plea to governments to reconsider policies regarding the safety of glyphosate residues in foods.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3945755/

2.1.2 Fórmulas comerciais de herbicidas à base de glifosato

As análises de risco devem levar em conta não apenas as propriedades tóxicas do glifosato e do seu principal produto de degradação (o ácido aminometilfosfônico – AMPA). O efeito conjugado dos demais componentes de caldas vendidas como "herbicidas a base de glifosato" (a exemplo do Roundup) também devem ser avaliados.

De fato, os adjuvantes presentes nas fórmulas comerciais não apenas carregam sua própria toxicidade como ainda podem influenciar sobre o grau de agressividade do princípio ativo. Trata-se de tema delicado, em que pese o acúmulo de evidências. As classificações toxicológicas aplicadas aos agrotóxicos resultam de estudos realizados tão somente com os princípios ativos, que constituem apenas parte dos produtos comerciais. Na prática os agricultores utilizarão fórmulas comerciais compostas, que impactarão os ambientes e as pessoas envolvidas de forma não correspondente ao que poderia ser estimado por meio de estudos focados em possíveis efeitos dos princípios ativos. Ocorre situação assemelhada na elaboração de índices de consumo diário aceitável (IDA) ou de Limites Máximos de Resíduo (LMR) tolerados em determinada circunstância, que também resultam de baixa eficácia para controlar danos. Essas considerações, que se aplicam tanto ao Roundup quanto a vários outros venenos agrícolas, revelam a necessidade de estudos mais completos e cuidadosos.

Mesnage, R.; Defarge, N.; Vendômois, J.; Séralini, G-E. 2014. Major pesticides are more toxic to human cells than their declared active principles. *Biomedical Research International*, 2014: 179691.

Pesticides are used throughout the world as mixtures called formulations. They contain adjuvants, which are often kept confidential and are called inerts by the manufacturing companies, plus a declared active principle (AP), which is usually tested alone. This is true even in the longest toxicological regulatory tests performed on mammals . We tested the toxicity of 9 pesticides, comparing active principles and their formulations, on three human cell lines (HepG2, HEK293 and JEG3). We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. Glyphosate, isoprot uron, fluroxypyr, pirimicarb, imidacloprid, acetamiprid, tebuc onazole, epoxiconazole and prochloraz constitute respectively the active principles of 3 major herbicides, 3 insecticides and 3 fungicides. Fungicides were the most toxic from concentrations 300-600 times lower than agricultural dilutions, followed by herbicides, and then insecticides, with very similar profiles in all cell types. The human placental JEG3 cells appeared to be the most sensitive. Despite its relatively benign reputation, Roundup was by far the most toxic among the herbicides and insecticides tested. Most importantly, 8 formulations out of 9 were several hundred times more toxic than their active principle. Our results challenge the relevance of the Acceptable Daily Intake for pesticides because this norm is calculated from the toxicity of the active principle alone. The study of combinatorial effects of several APs together may be of only secondary importance if the toxicity of the combinations of each AP with its adjuvants is neglected or unknown. Chronic tests on pesticides may not reflect relevant environmental exposures if only one ingredient of these mixtures is tested alone.

Artigo completo disponível em https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3955666/

No caso das formulações a base de glifosato, sabe-se que a polioxietilenamida (ou POEA, sigla em inglês) possui maior toxicidade do que o princípio ativo em si. Isso significa que o uso de herbicidas como o Roundup leva maiores riscos de toxicidade do que seria verificado em aplicações “puras”, isoladas, de seu princípio ativo, caso este viesse a ser usado dessa forma (como gostariam os estudiosos que defendem sua baixa agressividade).

Sawada, Y.; Nagai, Y.; Ueyama, M.; Yamarnoto, I. 1988. Probable toxicity of surface-active agent in commercial herbicide containing glyphosate. *The Lancet*, 1 (85-80):229.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/2893109

Marc, J.; Mulner-Lorillon, O.; Boulben, S.; Hureau, D.; Durand, G.; Bellé, R. 2002. Pesticide Roundup provokes cell division dysfunction at the level of CDK1/ cyclin B activation. *Chem Res Toxicol*, 15, 32631.

To assess human health risk from environmental chemicals, we have studied the effect on cell cycle regulation of the widely used glyphosate-containing pesticide Roundup. As a model system we have used sea urchin embryonic first divisions following fertilization, which are appropriate for the study of universal cell cycle regulation without interference with transcription. We show that 0.8% Roundup (containing 8 mM glyphosate) induces a delay in the kinetic of the first cell cleavage of sea urchin embryos. The delay is dependent on the concentration of Roundup. The delay in the cell cycle could be induced using increasing glyphosate concentrations (1-10 mM) in the presence of a subthreshold concentration of Roundup 0.2%, while glyphosate alone was ineffective, thus indicating synergy between glyphosate and Roundup formulation products. The effect of Roundup was not lethal and involved a delay in entry into M-phase of the cell cycle, as judged cytologically. Since CDK1/cyclin B regulates universally the M-phase of the cell cycle, we analyzed CDK1/cyclin B activation during the first division of early development. Roundup delayed the activation of CDK1/cyclin B in vivo. Roundup inhibited also the global protein synthetic rate without preventing the accumulation of cyclin B. In summary, Roundup affects cell cycle regulation by delaying activation of the CDK1/cyclin B complex, by synergic effect of glyphosate and formulation products. Considering the universality among species of the CDK1/cyclin B regulator, our results question the safety of glyphosate and Roundup on human health.

http://www.ncbi.nlm.nih.gov/pubmed/11896679

Richard, S.; Moslemi, S.; Siphautar, H.; Benachour, N.; Séralini, G-E. 2005. Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ Health Perspect.* 113 (6), 71620.

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1257596/

Martinez, A.; Reyes, I.; Reyes, N. 2007. Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells. *Biomédica*, 27(4):594-604.

Introduction: Glyphosate is a broad-spectrum, non-selective herbicide and commonly used to eliminate weeds in agricultural and forest settings. Studies evaluating glyphosate toxicity in animals and environment show that commercial formulations of glyphosate are more toxic than the active component itself.
Objectives: Technical grade glyphosate was compared with the commercial formulation Roundup

in their respective toxicities on human peripheral blood mononuclear cells.
Materials and Methods: Human peripheral blood mononuclear cells were exposed to different concentrations of glyphosate, either technical grade or in the form of Roundup for 24 h, 48 h, 72 h, and 96 h. Cytotoxicity was assayed by trypan blue dye exclusion method and reduction of (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2Htetrazolium-5-carboxyanilide inner salt)XTT reagent.
Results: Both technical grade glyphosate and Roundup formulation were toxic to human peripheral blood mononuclear cells. Cytotoxicity of Roundup was higher than cytotoxicity of glyphosate, since the LC50 (50% lethal concentration) determined by the trypan blue exclusion method at 24 h was the equivalent of 56.4 microg/ml of glyphosate in the form of Roundup and 1,640 microg/ml (1.64 mg/ml) for technical grade glyphosate.
Conclusions: This in vitro study confirmed the toxic effects on human cells by glyphosate and its commercial preparations. Commercial formulations were more cytotoxic than the active component alone, supporting the concept that additives in commercial formulations play a role in the toxicity attributed to glyphosate-based herbicides

Artigo completo disponível em http://www.scielo.org.co/scielo.php?pid=S0120-41572007000400014&script=sci_arttext

Koller, V.; Furhacker, M.; Nersesyan, A.; Mišík, M.; Eisenbauer, M.; Knasmueller, S. 2012. Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol*, 86 (5): 805-813.

Glyphosate (G) is the largest selling herbicide worldwide; the most common formulations (Roundup, R) contain polyoxyethyleneamine as main surfactant. Recent findings indicate that G exposure may cause DNA damage and cancer in humans. Aim of this investigation was to study the cytotoxic and genotoxic properties of G and R (UltraMax) in a buccal epithelial cell line (TR146), as workers are exposed via inhalation to the herbicide. R induced acute cytotoxic effects at concentrations > 40 mg/l after 20 min, which were due to membrane damage and impairment of mitochondrial functions. With G, increased release of extracellular lactate dehydrogenase indicative for membrane damage was observed at doses > 80 mg/l. Both G and R induced DNA migration in single-cell gel electrophoresis assays at doses > 20 mg/l. Furthermore, an increase of nuclear aberrations that reflect DNA damage was observed. The frequencies of micronuclei and nuclear buds were elevated after 20-min exposure to 10-20 mg/l, while nucleoplasmatic bridges were only enhanced by R at the highest dose (20 mg/l). R was under all conditions more active than its active principle (G). Comparisons with results of earlier studies with lymphocytes and cells from internal organs indicate that epithelial cells are more susceptible to the cytotoxic and DNA-damaging properties of the herbicide and its formulation. Since we found genotoxic effects after short exposure to concentrations that correspond to a 450-fold dilution of spraying used in agriculture, our findings indicate that inhalation may cause DNA damage in exposed individuals.

http://www.ncbi.nlm.nih.gov/pubmed/22331240

Mesnage, R.; Bernay, B.; Séralini, G-E. 2012. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*.

Pesticides are always used in formulations as mixtures of an active principle with adjuvants. Glyphosate, the active ingredient of the major pesticide in the world, is an herbicide supposed to be specific on plant metabolism. Its adjuvants are generally considered as inert diluents. Since side effects for all these compounds have been claimed, we studied potential active principles for toxicity on human cells for 9 glyphosate-based formulations. For this we detailed their compositions and toxicities, and as controls we used a major adjuvant (the polyethoxylated tallowamine POE-15), glyphosate alone, and a total formulation without glyphosate. This was performed after 24h

> exposures on hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines. We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. The compositions in adjuvants were analyzed by mass spectrometry. Here we demonstrate that all formulations are more toxic than glyphosate, and we separated experimentally three groups of formulations differentially toxic according to their concentrations in ethoxylated adjuvants. Among them, POE-15 clearly appears to be the most toxic principle against human cells, even if others are not excluded. It begins to be active with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3ppm, at environmental/occupational doses. We demonstrate in addition that POE-15 induces necrosis when its first micellization process occurs, by contrast to glyphosate which is known to promote endocrine disrupting effects after entering cells. Altogether, these results challenge the establishment of guidance values such as the acceptable daily intake of glyphosate, when these are mostly based on a long term in vivo test of glyphosate alone. Since pesticides are always used with adjuvants that could change their toxicity, the necessity to assess their whole formulations as mixtures becomes obvious. This challenges the concept of active principle of pesticides for non-target species.
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> Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2012/11/2012.-Mesnage-et-al.-Ethoxylated-adjuvants-of-glyphosate-based-herbicides-are-active-principles-of-human-cell-toxicity.pdf

A literatura científica atualizada é farta em estudos que registram aspectos de toxicidade dos herbicidas à base de glifosato. A maior parte deles refere-se a diferentes formulações de Roundup, fazendo-se mais adequados como suporte de decisão em termos de riscos (estudos do produto comercial superam em qualidade/representatividade as avaliações baseadas no princípio ativo).

Verifica-se nos artigos referenciados a seguir que esses herbicidas devem ser considerados citotóxicos, teratogênicos, cancerígenos e disruptores endócrinos, no médio e longo prazo. Esses danos se observam a partir de doses inferiores àquelas consideradas "sem risco" pela maioria dos órgãos reguladores (dimensionadas com base em estimativas de ingestão de resíduos contidos nos alimentos). Mais recentemente, propriedades antibióticas dos herbicidas à base de glifosato chamaram a atenção dos pesquisadores sobre as alterações provocadas na microbiota simbiótica dos mamíferos (inclusive o ser humano[56]), com impactos relevantes sobre indicadores de degradação da saúde humana/animal (e de degradação ambiental).

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[56] Vale lembrar do papel biológico fundamental dos micro-organismos simbiontes do ser humano sobre a saúde, tal como a qualidade do processo digestivo, a proteção da pele ou até a estabilidade neuroquimíca. De fato, o microbioma passa a ser considerado em alguns campos da ciência biomédica como um órgão do próprio corpo humano, e são várias as patologias que envolvem perturbações do microbioma (obesidade, doenças autoimunes e provavelmente cânceres).

Walsh, L.; McCormick, C.; Martin, C.; Stocco, D. 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environmental Health Perspectives*, 108, 769–776.

Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450scc) enzyme initiates the synthesis of all steroid hormones. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450scc enzyme (which converts cholesterol to pregnenolone) and the 3beta-hydroxysteroid dehydrogenase (3beta-HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyryl [(Bu)(2)]cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1638308/pdf/envhper00309-0125.pdf

Dallegrave, E.; Mantese, F.; Coelho, R.; Pereira, J.; Dalsenter, P.; Langeloh, A. 2003. The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. *Toxicol Lett*, 142, 45-52.

The aim of this study was to assess the teratogenicity of the herbicide glyphosate-Roundup (as commercialized in Brazil) to Wistar rats. Dams were treated orally with water or 500, 750 or 1000 mg/kg glyphosate from day 6 to 15 of pregnancy. Cesarean sections were performed on day 21 of pregnancy, and number of corpora lutea, implantation sites, living and dead fetuses, and resorptions were recorded. Weight and gender of the fetuses were determined, and fetuses were examined for external malformations and skeletal alterations. The organs of the dams were removed and weighed. Results showed a 50%, mortality rate for dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in 15.4, 33.1, 42.0 and 57.3% of fetuses from the control, 500, 750 and 1000 mg/kg glyphosate groups, respectively. We may conclude that glyphosate-Roundup is toxic to the dams and induces developmental retardation of the fetal skeleton.

http://www.ncbi.nlm.nih.gov/pubmed/12765238

Marc, J.; Bellé, R.; Morales, J.; Cormier, P.; Mulner-Lorillon, O. 2004. Formulated glyphosate activates the DNA-response checkpoint of the cell cycle leading to the prevention of G2/M transition. *Toxicological Sciences*, 82, 436-442.

A glyphosate containing pesticide impedes at 10 mM glyphosate the G2/M transition as judged from analysis of the first cell cycle of sea urchin development. We show that formulated glyphosate prevented dephosphorylation of Tyr 15 of the cell cycle regulator CDK1/cyclin B in vivo, the end point target of the G2/M cell cycle checkpoint. Formulated glyphosate had no direct effect on the dual specific cdc25 phosphatase activity responsible for Tyr 15 dephosphorylation. At a concentration that efficiently impeded the cell cycle, formulated glyphosate inhibited the synthesis of DNA occurring in S phase of the cell cycle. The extent of the inhibition of DNA synthesis by formulated glyphosate was correlated with the effect on the cell cycle. We conclude that formulated glyphosate's effect on the cell cycle is exerted at the level of the DNA-response checkpoint of S phase. The resulting inhibition of CDK1/cyclin B Tyr 15 dephosphorylation leads to prevention of the G2/M transition and cell cycle progression.

http://www.ncbi.nlm.nih.gov/pubmed/15375296

Marc, J.; Mulner-Lorillon, O.; Bellé, R. 2004. Glyphosate based pesticides affect cell cycle regulation. *Biology of the Cell*, 96: 245249.

Cell-cycle dysregulation is a hallmark of tumor cells and human cancers. Failure in the cell-cycle checkpoints leads to genomic instability and subsequent development of cancers from the initial affected cell. A worldwide used product Roundup 3plus, based on glyphosate as the active herbicide, was suggested to be of human health concern since it induced cell cycle dysfunction as judged from analysis of the first cell division of sea urchin embryos, a recognized model for cell cycle studies. Several glyphosate-based pesticides from different manufacturers were assayed in comparison with Roundup 3plus for their ability to interfere with the cell cycle regulation. All the tested products, Amega, Cargly, Cosmic, and Roundup Biovert induced cell cycle dysfunction. The threshold concentration for induction of cell cycle dysfunction was evaluated for each product and suggests high risk by inhalation for people in the vicinity of the pesticide handling sprayed at 500 to 4000 times higher dose than the cell-cycle adverse concentration.

http://www.ncbi.nlm.nih.gov/pubmed/15182708

Benedetti, A.; Vituri Cde, L.; Trentin, A; Domingues, M.; Alvarez-Silva, M. 2004. The effects of sub-chronic exposure of Wistar rats to the herbicide Glyphosate-Biocarb. *Toxicol Lett*, 153(2): 227–232.

The object of this study was to analyze the hepatic effects of the herbicide Glyphosate-Biocarb (as commercialized in Brazil) in Wistar rats. Animals were treated orally with water or 4.87, 48.7, or 487 mg/kg of glyphosate each 2 days, during 75 days. Sub-chronic treatment of animals starting from the lowest dose of glyphosate induced the leakage of hepatic intracellular enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible damage in hepatocytes. We observed the increase of Kupffer cells in hepatic sinusoid of glyphosate-treated animals. This was followed by large deposition of reticulin fibers, composed mainly of collagen type III. We may conclude that Glyphosate-Biocarb may induce hepatic histological changes as well as AST and ALT leaking from liver to serum in experimental models.

http://www.ncbi.nlm.nih.gov/pubmed/15451553

EPI - Roos, A.; Blair, A.; Rusiecki, J.; Hoppin, J.; Svec, M.; Dosemeci, M.; Sandler, D.; Alavanja, M. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study. *Environmental Health Perspectives*, Volume 113, Number 1.

Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from in vitro and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrollment (1993-1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as a) ever personally mixed or applied products containing glyphosate; b) cumulative lifetime days of use, or "cumulative exposure days" (years of use times days/year); and c) intensity-weighted cumulative exposure days (years of use times days/year times estimated intensity level). Poisson regression was used to estimate exposure-response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1253709/

EPI - Paz-y-Miño, C.; Sánchez, M.; Arévalo, M.; Muñoz, M.; Witte, T.; De-la- Carrera, G.; Leone, P. 2007. Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genetics and Molecular Biology*, 30, 456-460.

We analyzed the consequences of aerial spraying with glyphosate added to a surfactant solution in the northern part of Ecuador. A total of 24 exposed and 21 unexposed control individuals were investigated using the comet assay. The results showed a higher degree of DNA damage in the exposed group (comet length = 35.5 μm) compared to the control group (comet length = 25.94 μm). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.

Artigo completp disponível em http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-47572007000300026

Dallegrave, E; Mantese, F; Oliveira, R.; Andrade, A.; Dalsenter, P.; Langeloh, A. 2007. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. *Arch Toxicol*, 81(9):665-73.

Glyphosate is the active ingredient and polyoxyethyleneamine is the surfactant present in the herbicide Roundup formulation commercialized in Brazil. The aim of this study was to assess the reproductive effects of glyphosate-Roundup on male and female offspring of Wistar rats exposed during pregnancy and lactation. Dams were treated orally with water or 50, 150 or 450 mg/kg glyphosate during pregnancy (21-23 days) and lactation (21 days). These doses do not correspond to human exposure levels. The results showed that glyphosate-Roundup did not induce maternal toxicity but induced adverse reproductive effects on male offspring rats: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in the serum testosterone level at puberty, and signs of individual spermatid degeneration during both periods. There was only a vaginal canal-opening delay in the exposed female offspring. These findings suggest that in utero and lactational exposure to glyphosate-Roundup may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood.

http://www.ncbi.nlm.nih.gov/pubmed/17634926

Benachour, N.; Sipahutar, H.; Moslemi, S.; Gasnier, C.; Travert, C.; Séralini, G-E. 2007. Time- and dose- dependent effects of Roundup on human embryonic and placental cells. *Archives of Environmental Contamination and Toxicology*, 53, 126-33.

Roundup is the major herbicide used worldwide, in particular on genetically modified plants that have been designed to tolerate it. We have tested the toxicity and endocrine disruption potential of Roundup (Bioforce on human embryonic 293 and placental-derived JEG3 cells, but also on normal human placenta and equine testis. The cell lines have proven to be suitable to estimate hormonal activity and toxicity of pollutants. The median lethal dose (LD(50)) of Roundup with embryonic cells is 0.3% within 1 h in serum-free medium, and it decreases to reach 0.06% (containing among other compounds 1.27 mM glyphosate) after 72 h in the presence of serum. In these conditions, the embryonic cells appear to be 2-4 times more sensitive than the placental ones. In all instances, Roundup (generally used in agriculture at 1-2%, i.e., with 21-42 mM glyphosate) is more efficient than its active ingredient, glyphosate, suggesting a synergistic effect provoked by the adjuvants present in Roundup. We demonstrated that serum-free cultures, even on a short-term basis (1 h), reveal the xenobiotic impacts that are visible 1-2 days later in serum. We also document at lower non-overtly toxic doses, from 0.01% (with 210 microM glyphosate) in 24 h, that Roundup is an aromatase disruptor. The direct inhibition is temperature-dependent and is confirmed in different tissues and species (cell lines from placenta or embryonic kidney, equine testicular, or human fresh placental extracts). Furthermore, glyphosate acts directly as a partial inactivator on microsomal aromatase, independently of its acidity, and in a dose-dependent manner. The cytotoxic, and potentially endocrine-disrupting effects of Roundup are thus amplified with time. Taken together, these data suggest that Roundup exposure may affect human reproduction and fetal development in case of contamination. Chemical mixtures in formulations appear to be underestimated regarding their toxic or hormonal impact.

http://www.ncbi.nlm.nih.gov/pubmed/17486286

Bellé, R.; Le Bouffant, R.; Morales, J.; Cosson, B.; Cormier, P.; Mulner-Lorillon, O. 2007: Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development. *J Soc Biol*, 201, 317-327.

Cell division is an essential process for heredity, maintenance and evolution of the whole living kingdom. Sea urchin early development represents an excellent experimental model for the analysis of cell cycle checkpoint mechanisms since embryonic cells contain a functional DNA-damage checkpoint and since the whole sea urchin genome is sequenced. The DNA-damaged checkpoint is responsible for an arrest in the cell cycle when DNA is damaged or incorrectly replicated, for activation of the DNA repair mechanism, and for commitment to cell death by apoptosis in the case of failure to repair. New insights in cancer biology lead to two fundamental concepts about the very first origin of cancerogenesis. Cancers result from dysfunction of DNA-damaged checkpoints and cancers appear as a result of normal stem cell (NCS) transformation into a cancer stem cell (CSC). The second aspect suggests a new definition of "cancer", since CSC can be detected well before any clinical evidence. Since early development starts from the zygote, which is a primary stem cell, sea urchin early development allows analysis of the early steps of the cancerization process. Although sea urchins do not develop cancers, the model is alternative and complementary to stem cells which are not easy to isolate, do not divide in a short time and do not divide synchronously. In the field of toxicology and incidence on human health, the sea urchin experimental model allows assessment of cancer risk from single or combined molecules long before any epidemiologic evidence is available. Sea urchin embryos were used to test the worldwide used pesticide Roundup that contains glyphosate as the active herbicide agent; it was shown to activate the DNA-damage checkpoint of the first cell cycle of development. The model therefore allows considerable increase in risk evaluation of new products in the field of cancer and offers a tool for the discovery of molecular markers for early diagnostic in cancer biology. Prevention and early diagnosis are two decisive elements of human cancer therapy.

http://www.ncbi.nlm.nih.gov/pubmed/18157084

Cavaş, T.; Könen, S. 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, vol. 22 no. 4 pp. 263–268.

Glyphosate is a widely used broad-spectrum weed control agent. In the present study, an in vivo study on the genotoxic effects of a technical herbicide (Roundup) containing isopropylamine salt of glyphosate was carried out on freshwater goldfish Carassius auratus. The fish were exposed to three doses of glyphosate formulation (5, 10 and 15 ppm). Cyclophosphamide at a single dose of 5 mg/l was used as positive control. Analysis of micronuclei, nuclear abnormalities and DNA damage were performed on peripheral erythrocytes sampled at intervals of 48, 96 and 144 h posttreatment. Our results revealed significant dose-dependent increases in the frequencies of micronuclei, nuclear abnormalities as well as DNA strand breaks. Our findings also confirmed that the alkaline comet assay and nuclear deformations in addition to micronucleus test on fish erythrocytes in vivo are useful tools in determining the potential genotoxicity of commercial herbicides.

http://www.ncbi.nlm.nih.gov/pubmed/17426049

Malatesta, M.; Perdoni, F.; Santin, G.; Battistelli, S.; Muller, S.; Biggiogerra, M. 2008. Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicol in Vitro*, 22, 1853-1860.

Previous studies on mice fed genetically modified (GM) soybean demonstrated modifications of the mitochondrial functions and of the transcription/splicing pathways in hepatocytes. The cause(s) of these alterations could not be conclusively established but, since the GM soybean used is tolerant to glyphosate and was treated with the glyphosate-containing herbicide Roundup , the possibility exists that the effects observed may be due to herbicide residues. In order to verify this hypothesis, we treated HTC cells with 1-10mM Roundup and analysed cellular features by flow cytometry, fluorescence and electron microscopy. Under these experimental conditions, the death rate and the general morphology of HTC cells were not affected, as well as most of the cytoplasmic organelles. However, in HTC-treated cells, lysosome density increased and mitochondrial membranes modified indicating a decline in the respiratory activity. Moreover, nuclei underwent morpho-functional modifications suggestive of a decreased transcriptional/splicing activity. Although we cannot exclude that other factors than the presence of the herbicide residues could be responsible for the cellular modifications described in GM-fed mice, the concordance of the effects induced by low concentrations of Roundup on HTC cells suggests that the presence of Roundup residues could be one of the factors interfering with multiple metabolic pathways.

http://www.ncbi.nlm.nih.gov/pubmed/18835430

Prasad, S.; Srivastava, S.; Singh, M.; Shukla, Y. 2009. Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice. *Journal of Toxicology*, Article ID 308985, 6 pages.

Glyphosate (N-(phosphonomethyl) glycine, C(3)H(8)NO(5)P), a herbicide, used to control unwanted annual and perennial plants all over the world. Nevertheless, occupational and environmental exposure to pesticides can pose a threat to nontarget species including human beings. Therefore, in the present study, genotoxic effects of the herbicide glyphosate were analyzed by measuring chromosomal aberrations (CAs) and micronuclei (MN) in bone marrow cells of Swiss albino mice. A single dose of glyphosate was given intraperitoneally (i.p) to the animals at a concentration of 25 and 50 mg/kg b.wt. Animals of positive control group were injected i.p. benzo(a)pyrene (100 mg/kg b.wt., once only), whereas, animals of control (vehicle) group were injected i.p. dimethyl sulfoxide (0.2 mL). Animals from all the groups were sacrificed at sampling times of 24, 48, and 72 hours and their bone marrow was analyzed for cytogenetic and chromosomal damage. Glyphosate treatment significantly increases CAs and MN induction at both treatments and time compared with the vehicle control ($P < .05$). The cytotoxic effects of glyphosate were also evident, as observed by significant decrease in mitotic index (MI). The present results indicate that glyphosate is clastogenic and cytotoxic to mouse bone marrow.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2809416/

Gasnier, C.; Dumont, C.; Benachour, N.; Clair, E.; Chagnon, M-C.; Séralini, G-E. 2009. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, 262, 184191.

Glyphosate-based herbicides are the most widely used across the world; they are commercialized in different formulations. Their residues are frequent pollutants in the environment. In addition, these herbicides are spread on most eaten transgenic plants, modified to tolerate high levels of these compounds in their cells. Up to 400 ppm of their residues are accepted in some feed. We exposed human liver HepG2 cells, a well-known model to study xenobiotic toxicity, to four different formulations and to glyphosate, which is usually tested alone in chronic in vivo regulatory studies. We measured cytotoxicity with three assays (Alamar Blue, MTT, ToxiLight), plus genotoxicity (comet assay), anti-estrogenic (on ERalpha, ERbeta) and anti-androgenic effects (on AR) using gene reporter tests. We also checked androgen to estrogen conversion by aromatase activity and mRNA. All parameters were disrupted at sub-agricultural doses with all formulations within 24h. These effects were more dependent on the formulation than on the glyphosate concentration. First, we observed a human cell endocrine disruption from 0.5 ppm on the androgen receptor in MDA-MB453-kb2 cells for the most active formulation (R400), then from 2 ppm the transcriptional activities on both estrogen receptors were also inhibited on HepG2. Aromatase transcription and activity were disrupted from 10 ppm. Cytotoxic effects started at 10 ppm with Alamar Blue assay (the most sensitive), and DNA damages at 5 ppm. A real cell impact of glyphosate-based herbicides residues in food, feed or in the environment has thus to be considered, and their classifications as carcinogens/mutagens/reprotoxics is discussed.

Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2013/01/Gasnieral.TOX_2009.pdf

Benachour, N.; Séralini, G-E. 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chemical Research in Toxicology*, 22(1): 97-105.

We have evaluated the toxicity of four glyphosate (G)-based herbicides in Roundup (R) formulations, from 10^5 times dilutions, on three different human cell types. This dilution level is far below agricultural recommendations and corresponds to low levels of residues in food or feed. The formulations have been compared to G alone and with its main metabolite AMPA or with one known adjuvant of R formulations, POEA. HUVEC primary neonate umbilical cord vein cells have been tested with 293 embryonic kidney and JEG3 placental cell lines. All R formulations cause total cell death within 24 h, through an inhibition of the mitochondrial succinate dehydrogenase activity, and necrosis, by release of cytosolic adenylate kinase measuring membrane damage. They also induce apoptosis via activation of enzymatic caspases 3/7 activity. This is confirmed by characteristic DNA fragmentation, nuclear shrinkage (pyknosis), and nuclear fragmentation (karyorrhexis), which is demonstrated by DAPI in apoptotic round cells. G provokes only apoptosis, and HUVEC are 100 times more sensitive overall at this level. The deleterious effects are not proportional to G concentrations but rather depend on the nature of the adjuvants. AMPA and POEA separately and synergistically damage cell membranes like R but at different concentrations. Their mixtures are generally even more harmful with G. In conclusion, the R adjuvants like POEA change human cell permeability and amplify toxicity induced already by G, through apoptosis and necrosis. The real threshold of G toxicity must take into account the presence of adjuvants but also G metabolism and time-amplified effects or bioaccumulation. This should be discussed when analyzing the in vivo toxic actions of R. This work clearly confirms that the adjuvants in Roundup formulations are not inert. Moreover, the proprietary mixtures available on the market could cause cell damage and even death around residual levels to be expected, especially in food and feed derived from R formulation-treated crops.

http://pubs.acs.org/doi/abs/10.1021/tx800218n

George, J.; Prasad, S.; Mahmood, Z.; Shukla, Y. 2010. Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach. *J Proteomics*, 73(5), 951-964.

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu-Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu-Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

http://www.ncbi.nlm.nih.gov/pubmed/20045496

Paganelli, A.; Gnazzo, V.; Acosta, H.; López, S.; Carrasco, A. 2010. Glyphosate based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chemical Research in Toxicology*.

The broad spectrum herbicide glyphosate is widely used in agriculture worldwide. There has been ongoing controversy regarding the possible adverse effects of glyphosate on the environment and on human health. Reports of neural defects and craniofacial malformations from regions where glyphosate-based herbicides (GBH) are used led us to undertake an embryological approach to explore the effects of low doses of glyphosate in development.*Xenopus laevis* embryos were incubated with 1/5000 dilutions of a commercial GBH. The treated embryos were highly abnormal with marked alterations in cephalic and neural crest development and shortening of the anterior–posterior (A-P) axis. Alterations on neural crest markers were later correlated with deformities in the cranial cartilages at tadpole stages. Embryos injected with pure glyphosate showed very similar phenotypes. Moreover, GBH produced similar effects in chicken embryos, showing a gradual loss of rhombomere domains, reduction of the optic vesicles, and microcephaly. This suggests that glyphosate itself was responsible for the phenotypes observed, rather than a surfactant or other component of the commercial formulation. A reporter gene assay revealed that GBH treatment increased endogenous retinoic acid (RA) activity in *Xenopus* embryos and cotreatment with a RA antagonist rescued the teratogenic effects of the GBH. Therefore, we conclude that the phenotypes produced by GBH are mainly a consequence of the increase of endogenous retinoid activity. This is consistent with the decrease of Sonic hedgehog (Shh) signaling from the embryonic dorsal midline, with the inhibition of *otx2* expression and with the disruption of cephalic neural crest development.

The direct effect of glyphosate on early mechanisms of morphogenesis in vertebrate embryos opens concerns about the clinical findings from human offspring in populations exposed to GBH in agricultural fields.

http://pubs.acs.org/doi/abs/10.1021/tx1001749

Romano, R.; Romano, M.; Bernardi, M.; Furtado, P.; Oliveira, C. 2010. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. *Arch Toxicol*, 84 (4), 309–317.

Glyphosate is a herbicide widely used to kill weeds both in agricultural and non-agricultural landscapes. Its reproductive toxicity is related to the inhibition of a StAR protein and an aromatase enzyme, which causes an in vitro reduction in testosterone and estradiol synthesis. Studies in vivo about this herbicide effects in prepubertal Wistar rats reproductive development were not performed at this moment. Evaluations included the progression of puberty, body development, the hormonal production of testosterone, estradiol and corticosterone, and the morphology of the testis. Results showed that the herbicide (1) significantly changed the progression of puberty in a dose-dependent manner; (2) reduced the testosterone production, in semineferous tubules' morphology, decreased significantly the epithelium height (P < 0.001; control = 85.8 +/- 2.8 microm; 5 mg/kg = 71.9 +/- 5.3 microm; 50 mg/kg = 69.1 +/- 1.7 microm; 250 mg/kg = 65.2 +/- 1.3 microm) and increased the luminal diameter (P < 0.01; control = 94.0 +/- 5.7 microm; 5 mg/kg = 116.6 +/- 6.6 microm; 50 mg/kg = 114.3 +/- 3.1 microm; 250 mg/kg = 130.3 +/- 4.8 microm); (4) no difference in tubular diameter was observed; and (5) relative to the controls, no differences in serum corticosterone or estradiol levels were detected, but the concentrations of testosterone serum were lower in all treated groups (P < 0.001; control = 154.5 +/- 12.9 ng/dL; 5 mg/kg = 108.6 +/- 19.6 ng/dL; 50 mg/dL = 84.5 +/- 12.2 ng/dL; 250 mg/kg = 76.9 +/- 14.2 ng/dL). These results suggest that commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats when the exposure was performed during the puberty period.

http://www.ncbi.nlm.nih.gov/pubmed/20012598

Shehata, A.; Schrödl, W.; Aldin, A.; Hafez, H.; Krüger, M. 2012. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota *In Vitro*. *Curr Microbiol*, DOI 10.1007/s00284-012-0277-2.

The use of glyphosate modifies the environment which stresses the living microorganisms. The aim of the present study was to determine the real impact of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. The presented results evidence that the highly pathogenic bacteria as Salmonella Entritidis, Salmonella Gallinarum, Salmonella Typhimurium, Clostridium perfringens and Clostridium botulinum are highly resistant to glyphosate. However, most of beneficial bacteria as Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis and Lactobacillus spp. were found to be moderate to highly susceptible. Also Campylobacter spp. were found to be susceptible to glyphosate. A reduction of beneficial bacteria in the gastrointestinal tract microbiota by ingestion of glyphosate could disturb the normal gut bacterial community. Also, the toxicity of glyphosate to the most prevalent Enterococcus spp. could be a significant predisposing factor that is associated with the increase in C. botulinum-mediated diseases by suppressing the antagonistic effect of these bacteria on clostridia.

Artigo completo disponível em http://www.netwerkvlv.nl/downloads/2012-Krueger,%20M-glyphosate%20effects.pdf

Jasper, R.; Locatelli, G.; Pilati, C.; Locatelli, C. 2012. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. *Interdisciplinary Toxicology*, 5(3): 133–140, doi: 10.2478/v10102-012-0022-5.

We evaluated the toxicity of hepatic, hematological, and oxidative effects of glyphosate-Roundup® on male and female albino Swiss mice. The animals were treated orally with either 50 or 500 mg/kg body weight of the herbicide, on a daily basis for a period of 15 days. Distilled water was used as control treatment. Samples of blood and hepatic tissue were collected at the end of the treatment. Hepatotoxicity was monitored by quantitative analysis of the serum enzymes ALT, AST, and γ-GT and renal toxicity by urea and creatinine. We also investigated liver tissues histopathologically. Alterations of hematological parameters were monitored by RBC, WBC, hemoglobin, hematocrit, MCV, MCH, and MCHC. TBARS (thiobarbituric acid reactive substances) and NPSH (non-protein thiols) were analyzed in the liver to assess oxidative damage. Significant increases in the levels of hepatic enzymes (ALT, AST, and γ-GT) were observed for both herbicide treatments, but no considerable differences were found by histological analysis. The hematological parameters showed significant alterations (500 mg/kg body weight) with reductions of RBC, hematocrit, and hemoglobin, together with a significant increase of MCV, in both sexes of mice. In males, there was an important increase in lipid peroxidation at both dosage levels, together with an NPSH decrease in the hepatic tissue, whereas in females significant changes in these parameters were observed only at the higher dose rate. The results of this study indicate that glyphosate-Roundup® can promote hematological and hepatic alterations, even at subacute exposure, which could be related to the induction of reactive oxygen species.

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Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3600513/

REV – Antoniou, M.; Habib, M.; Howard, C.; Jennings, R.; Nodari, R.; Robinson, C.; Fagan, J. 2012. Teratogenic effects of glyphosate-based herbicides: Divergence of regulatory decisions from scientific evidence. *Journal of Environmental & Analytical Toxicology*, S4:006.

The publication of a study in 2010 showing that a glyphosate herbicide formulation and glyphosate alone caused malformations in the embryos of Xenopus laevis and chickens caused a scientific and political controversy. Debate centred on the effects of the production and consumption of genetically modified Roundup® Ready® soy, which is engineered to tolerate applications of glyphosate herbicide. This study, along with others indicating teratogenic and reproductive effects from glyphosate herbicide exposure, was rebutted by the German Federal Office for Consumer Protection and Food Safety, BVL, as well as in industry-sponsored papers. These rebuttals relied partly on unpublished industry-sponsored studies commissioned for regulatory purposes, which, it was claimed, showed that glyphosate is not teratogenic or a reproductive toxin. However, examination of the German authorities' draft assessment report (DAR) on the industry studies, which underlies glyphosate's EU authorisation, revealed further evidence of glyphosate's teratogenicity. Nevertheless, the German and EU authorities minimized these findings in their assessment and set a potentially unsafe acceptable daily intake (ADI) level for glyphosate. This paper reviews the evidence on the teratogenicity and reproductive toxicity of glyphosate herbicides and concludes that a new and transparent risk assessment needs to be conducted by scientists who are independent of industry and of the regulatory bodies that were involved in the existing authorisation of glyphosate.

Artigo completo disponível em http://omicsonline.org/teratogenic-effects-of-glyphosate-based-herbicides-divergence-of-regulatory-decisions-from-scientific-evidence-2161-0525.S4-006.php?aid=7453

Romano, M.; Romano, R.; Santos, L.; Wisniewski, P.; Campos, D.A; Souza, P.; Viau, P.; Bernardi, M.; Nunes, M.; Oliveira, C. 2012. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. *Arch Toxicol*, 86 (4), 663–673.

Sexual differentiation in the brain takes place from late gestation to the early postnatal days. This is dependent on the conversion of circulating testosterone into estradiol by the enzyme aromatase. The glyphosate was shown to alter aromatase activity and decrease serum testosterone concentrations. Thus, the aim of this study was to investigate the effect of gestational maternal glyphosate exposure (50 mg/kg, NOAEL for reproductive toxicity) on the reproductive development of male offspring. Sixty-day-old male rat offspring were evaluated for sexual behavior and partner preference; serum testosterone concentrations, estradiol, FSH and LH; the mRNA and protein content of LH and FSH; sperm production and the morphology of the seminiferous epithelium; and the weight of the testes, epididymis and seminal vesicles. The growth, the weight and age at puberty of the animals were also recorded to evaluate the effect of the treatment. The most important findings were increases in sexual partner preference scores and the latency time to the first mount; testosterone and estradiol serum concentrations; the mRNA expression and protein content in the pituitary gland and the serum concentration of LH; sperm production and reserves; and the height of the germinal epithelium of seminiferous tubules. We also observed an early onset of puberty but no effect on the body growth in these animals. These results suggest that maternal exposure to glyphosate disturbed the masculinization process and promoted behavioral changes and histological and endocrine problems in reproductive parameters. These changes associated with the hypersecretion of androgens increased gonadal activity and sperm production.

http://www.ncbi.nlm.nih.gov/pubmed/22120950

Clair, E.; Mesnage, R.; Travert, C.; Séralini, G-E. 2012. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. *Toxicol In Vitro*, 26 (2), 269–279.

The major herbicide used worldwide, Roundup, is a glyphosate-based pesticide with adjuvants. Glyphosate, its active ingredient in plants and its main metabolite (AMPA) are among the first contaminants of surface waters. Roundup is being used increasingly in particular on genetically modified plants grown for food and feed that contain its residues. Here we tested glyphosate and its formulation on mature rat fresh testicular cells from 1 to 10000ppm, thus from the range in some human urine and in environment to agricultural levels. We show that from 1 to 48h of Roundup exposure Leydig cells are damaged. Within 24-48h this formulation is also toxic on the other cells, mainly by necrosis, by contrast to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower non toxic concentrations of Roundup and glyphosate (1ppm), the main endocrine disruption is a testosterone decrease by 35%. The pesticide has thus an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested, and only with glyphosate in regulatory tests.

http://www.ncbi.nlm.nih.gov/pubmed/22200534

Séralini, G-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Vendomois, J. 2012. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food Chem Toxicol*, 50, 4221– 4231.

Retracted.

http://www.ncbi.nlm.nih.gov/pubmed/24490213

COM – Séralini, G-E.; Mesnage, R.; Defarge, N.; Gress, S.; Hennequin, D.; Clair, E.; Malatesta, M.; Vendômois, J. 2013. Answers to critics: Why there is a long term toxicity due to a Roundup-tolerant genetically modified maize and to a Roundup herbicide. *Food and Chemical Toxicology*, 53: 476-83.

Our recent work (Séralini et al., 2012) remains to date the most detailed study involving the life-long consumption of an agricultural genetically modified organism (GMO). This is true especially for NK603 maize for which only a 90-day test for commercial release was previously conducted using the same rat strain (Hammond et al., 2004). It is also the first long term detailed research on mammals exposed to a highly diluted pesticide in its total formulation with adjuvants. This may explain why 75% of our first criticisms arising within a week, among publishing authors, come from plant biologists, some developing patents on GMOs, even if it was a toxicological paper on mammals, and from Monsanto Company who owns both the NK603 GM maize and Roundup herbicide (R). Our study has limits like any one, and here we carefully answer to all criticisms from agencies, consultants and scientists, that were sent to the Editor or to ourselves. At this level, a full debate is biased if the toxicity tests on mammals of NK603 and R obtained by Monsanto Company remain confidential and thus unavailable in an electronic format for the whole scientific community to conduct independent scrutiny of the raw data. In our article, the conclusions of long-term NK603 and Roundup toxicities came from the statistically highly discriminant findings at the biochemical level in treated groups in comparison to controls, because these findings do correspond in an blinded analysis to the pathologies observed in organs, that were in turn linked to the deaths by anatomopathologists. GM NK603 and R cannot be regarded as safe to date.

Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2013/01/Seralinial-AnswersCritics-FCT_2013.pdf

Cavalli, V.; Cattani, D.; Rieg, C.; Pierozan, P.; Zanatta, L.; Parisotto, E.; Filho, D.; Silva, F.; Pessoa-Pureur, R.; Zamoner, A. 2013. Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. *Free Radical Biology and Medicine*, 65 (2013) 335–346.

Glyphosate is the primary active constituent of the commercial pesticide Roundup. The present results show that acute Roundup exposure at low doses (36 ppm, 0.036 g/L) for 30 min induces oxidative stress and activates multiple stress-response pathways leading to Sertoli cell death in prepubertal rat testis. The pesticide increased intracellular Ca(2+) concentration by opening L-type voltage-dependent Ca(2+) channels as well as endoplasmic reticulum IP3 and ryanodine receptors, leading to Ca(2+) overload within the cells, which set off oxidative stress and necrotic cell death. Similarly, 30 min incubation of testis with glyphosate alone (36 ppm) also increased (45)Ca(2+) uptake. These events were prevented by the antioxidants Trolox and ascorbic acid. Activated protein kinase C, phosphatidylinositol 3-kinase, and the mitogen-activated protein kinases such as ERK1/2 and p38MAPK play a role in eliciting Ca(2+) influx and cell death. Roundup decreased the levels of reduced glutathione (GSH) and increased the amounts of thiobarbituric acid-reactive species (TBARS) and protein carbonyls. Also, exposure to glyphosate-Roundup stimulated the activity of glutathione peroxidase, glutathione reductase, glutathione S-transferase, γ-glutamyltransferase, catalase, superoxide dismutase, and glucose-6-phosphate dehydrogenase, supporting downregulated GSH levels. Glyphosate has been described as an endocrine disruptor affecting the male reproductive system; however, the molecular basis of its toxicity remains to be clarified. We propose that Roundup toxicity, implicated in Ca(2+) overload, cell signaling misregulation, stress response of the endoplasmic reticulum, and/or depleted antioxidant defenses, could contribute to Sertoli cell disruption in spermatogenesis that could have an impact on male fertility.

http://www.ncbi.nlm.nih.gov/pubmed/23820267

George, J.; Shukla, Y. 2013. Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCaT cells. *ISRN Dermatology*, Volume 2013, Article ID 825180, 12 pages.

We demonstrated that glyphosate possesses tumor promoting potential in mouse skin carcinogenesis and SOD 1, calcyclin (S100A6), and calgranulin B (S100A9) have been associated with this potential, although the mechanism is unclear. We aimed to clarify whether imbalance in between $[Ca^{2+}]_i$ levels and oxidative stress is associated with glyphosate-induced proliferation in human keratinocytes HaCaT cells. The $[Ca^{2+}]_i$ levels, ROS generation, and expressions of G1/S cyclins, IP_3R1, S100A6, S100A9, and SOD 1, and apoptosis-related proteins were investigated upon glyphosate exposure in HaCaT cells. Glyphosate (0.1 mM) significantly induced proliferation, decreases $[Ca^{2+}]_i$, and increases ROS generation in HaCaT cells, whereas antioxidant N-acetyl-L-cysteine (NAC) pretreatment reverts these effects which directly indicated that glyphosate induced cell proliferation by lowering $[Ca^{2+}]_i$ levels via ROS generation. Glyphosate also enhanced the expression of G1/S cyclins associated with a sharp decrease in G0/G1 and a corresponding increase in S-phases. Additionally, glyphosate also triggers S100A6/S100A9 expression and decreases IP_3R1 and SOD 1 expressions in HaCaT cells. Notably, Ca^{2+}suppression also prevented apoptotic related events including Bax/Bcl-2 ratio and caspases activation. This study highlights that glyphosate promotes proliferation in HaCaT cells probably by disrupting the balance in between $[Ca^{2+}]_i$ levels and oxidative stress which in turn facilitated the downregulation of mitochondrial apoptotic signaling pathways.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3773425/#!po=65.3846

Séralini, G-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Vendômois, J. 2014**.** Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26:14.

Background: The health effects of a Roundup-tolerant NK603 genetically modified (GM) maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 ppb of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in rats. This study constitutes a follow-up investigation of a 90-day feeding study conducted by Monsanto in order to obtain commercial release of this GMO, employing the same rat strain and analyzing biochemical parameters on the same number of animals per group as our investigation. Our research represents the first chronic study on these substances, in which all observations including tumors are reported chronologically. Thus, it was not designed as a carcinogenicity study. We report the major findings with 34 organs observed and 56 parameters analyzed at 11 time points for most organs.
Results: Biochemical analyses confirmed very significant chronic kidney deficiencies, for all treatments and both sexes; 76% of the altered parameters were kidney-related. In treated males, liver congestions and necrosis were 2.5 to 5.5 times higher. Marked and severe nephropathies were also generally 1.3 to 2.3 times greater. In females, all treatment groups showed a two- to threefold increase in mortality, and deaths were earlier. This difference was also evident in three male groups fed with GM maize. All results were hormone- and sex-dependent, and the pathological profiles were comparable. Females developed large mammary tumors more frequently and before controls;

the pituitary was the second most disabled organ; the sex hormonal balance was modified by consumption of GM maize and Roundup treatments. Males presented up to four times more large palpable tumors starting 600 days earlier than in the control group, in which only one tumor was noted. These results may be explained by not only the non-linear endocrine-disrupting effects of Roundup but also by the overexpression of the EPSPS transgene or other mutational effects in the GM maize and their metabolic consequences.
Conclusion: Our findings imply that long-term (2 year) feeding trials need to be conducted to thoroughly evaluate the safety of GM foods and pesticides in their full commercial formulations.

Artigo completo disponível em http://www.enveurope.com/content/26/1/14

Koller, V.; Fürhacker, M.; Nersesyan, A.; Mišík, M.; Eisenbauer, M.; Knasmueller, S. 2014. Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol*, 86(5):805-13. doi: 10.1007/s00204-012-0804-8.

Glyphosate (G) is the largest selling herbicide worldwide; the most common formulations (Roundup, R) contain polyoxyethyleneamine as main surfactant. Recent findings indicate that G exposure may cause DNA damage and cancer in humans. Aim of this investigation was to study the cytotoxic and genotoxic properties of G and R (UltraMax) in a buccal epithelial cell line (TR146), as workers are exposed via inhalation to the herbicide. R induced acute cytotoxic effects at concentrations > 40 mg/l after 20 min, which were due to membrane damage and impairment of mitochondrial functions. With G, increased release of extracellular lactate dehydrogenase indicative for membrane damage was observed at doses > 80 mg/l. Both G and R induced DNA migration in single-cell gel electrophoresis assays at doses > 20 mg/l. Furthermore, an increase of nuclear aberrations that reflect DNA damage was observed. The frequencies of micronuclei and nuclear buds were elevated after 20-min exposure to 10-20 mg/l, while nucleoplasmatic bridges were only enhanced by R at the highest dose (20 mg/l). R was under all conditions more active than its active principle (G). Comparisons with results of earlier studies with lymphocytes and cells from internal organs indicate that epithelial cells are more susceptible to the cytotoxic and DNA-damaging properties of the herbicide and its formulation. Since we found genotoxic effects after short exposure to concentrations that correspond to a 450-fold dilution of spraying used in agriculture, our findings indicate that inhalation may cause DNA damage in exposed individuals.

http://www.ncbi.nlm.nih.gov/pubmed/22331240

EPI - Swanson, N.; Leu, A.; Abrahamson, J.; Wallet, B. 2014. Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. *Journal of Organic Systems*, 9(2).

A huge increase in the incidence and prevalence of chronic diseases has been reported in the United States (US) over the last 20 years. Similar increases have been seen globally. The herbicide glyphosate was introduced in 1974 and its use is accelerating with the advent of herbicide-tolerant genetically engineered (GE) crops. Evidence is mounting that glyphosate interferes with many metabolic processes in plants and animals and glyphosate residues have been detected in both. Glyphosate disrupts the endocrine system and the balance of gut bacteria, it damages DNA and is a driver of mutations that lead to cancer.
In the present study, US government databases were searched for GE crop data, glyphosate application data and disease epidemiological data. Correlation analyses were then performed on a total of 22 diseases in these time-series data sets. The Pearson correlation coefficients are highly significant (< 10-5) between glyphosate applications and hypertension (R = 0.923), stroke (R = 0.925), diabetes prevalence (R = 0.971), diabetes incidence (R = 0.935), obesity (R = 0.962), lipoprotein metabolism disorder (R = 0.973), Alzheimer's (R = 0.917), senile dementia (R = 0.994), Parkinson's (R = 0.875), multiple sclerosis (R = 0.828), autism (R = 0.989), inflammatory bowel disease (R = 0.938), intestinal infections (R = 0.974), end stage renal disease (R = 0.975), acute

kidney failure (R = 0.978), cancers of the thyroid (R = 0.988), liver (R = 0.960), bladder (R = 0.981), pancreas (R = 0.918), kidney (R = 0.973) and myeloid leukaemia (R = 0.878).
The Pearson correlation coefficients are highly significant (< 10-4) between the percentage of GE corn and soy planted in the US and hypertension (R = 0.961), stroke (R = 0.983), diabetes prevalence (R = 0.983), diabetes incidence (R = 0.955), obesity (R = 0.962), lipoprotein metabolism disorder (R = 0.955), Alzheimer's (R = 0.937), Parkinson's (R = 0.952), multiple sclerosis (R = 0.876), hepatitis C (R = 0.946), end stage renal disease (R = 0.958), acute kidney failure (R = 0.967), cancers of the thyroid (R = 0.938), liver (R = 0.911), bladder (R = 0.945), pancreas (R = 0.841), kidney (R = 0.940) and myeloid leukaemia (R = 0.889). The significance and strength of the correlations show that the effects of glyphosate and GE crops on human health should be further investigated.

Artigo completo disponível em http://www.organic-systems.org/journal/92/JOS_Volume-9_Number-2_Nov_2014-Swanson-et-al.pdf

Por ser um poluente com alta carga de presença, que pode ser encontrado no solo, na água, no ar e em diversos alimentos (ver referências contidas na Parte 3 item 2.1.1), o glifosato constitui um xenobiótico[57] de grande relevância, observado em tecidos e líquidos biológicos de organismos humanos e animais. Mesmo a barreira placentária se mostra permeável ao glifosato, facilitando a contaminação dos fetos e ameaçando a estabilidade de características transmitidas entre gerações.

Poulsen, M.; Rytting, E.; Mose, T.; Knudsen, L. 2009. Modeling placental transport: Correlation of *in vitro* BeWo cell permeability and ex vivo human placental perfusion. *Toxicol In Vitro*, 23: 1380–1386.

The placental passage of three compounds with different physicochemical properties was recently investigated in ex vivo human placental perfusion experiments (caffeine, benzoic acid, and glyphosate) [Mose, T., Kjaerstad, M.B., Mathiesen, L., Nielsen, J.B., Edelfors, S., Knudsen, L.E., 2008. Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system. J. Toxicol. Environ. Health, Part A 71, 984-991]. In this work, the transport of these same three compounds, plus the reference compound antipyrine, was investigated using BeWo (b30) cell monolayers. Transport across the BeWo cells was observed in the rank order of caffeine>antipyrine>benzoic acid>glyphosate in terms of both the apparent permeability coefficient and the initial slope, defined as the linear rate of substance transferred to the fetal compartment as percent per time, a parameter used to compare the two experimental models. The results from the in vitro studies were in excellent agreement with the ex vivo results (caffeine approximately antipyrine>benzoic acid>glyphosate). However the transfer rate was much slower in the BeWo cells compared to the perfusion system. The advantages and limitations of each model are discussed in order to assist in the preparation, prediction, and performance of future studies of maternal-fetal transfer.

http://www.ncbi.nlm.nih.gov/pubmed/19647068

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[57] Xenobióticos são compostos químicos estranhos a determinado organismo ou sistema biológico, onde podem ser encontrados mesmo quando não são ali produzidos ou esperados. O termo também indica substâncias presentes em concentrações muito mais elevadas do que o nível normal, a exemplo do que ocorreria em superdosagens de medicamentos que não fazem parte da dieta dos organismos onde são observados.

Aris, A.; Leblanc, S. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, doi:10.1016/j.reprotox.2011.02.004.

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

http://www.ncbi.nlm.nih.gov/pubmed/21338670

Krüger, M.; Schrödl, W.; Neuhaus, J.; Shehata, A. 2013. Field investigations of glyphosate in urine of Danish dairy cows. *J Environ Anal Toxicol*, 3(5): 100186.

In the present study, thirty dairy cows from each of eight Danish dairy farms were investigated for excretion of glyphosate in urine. Blood serum parameters indicative of cytotoxicity as alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), glutamate oxaloacetate transaminase (GOT), creatinine kinase CK), nephrotoxicity, (urea, creatine), cholesterol and the trace elements as manganese (Mn), cobalt (Co), selenium (Se), copper (Cu) and zinc (Zn) were investigated. All cows excreted glyphosate in their urine but in varying concentrations. Increased levels of GLDH, GOT and CK in cows from all farms demonstrate a possible effect of glyphosate on liver and muscle cells. High urea levels in some farms could be due to nephrotoxicity of glyphosate. Also the unexpected very low levels of Mn and Co were observed in all animals which could be explained due to a strong mineral chelating effect of glyphosate. In contrast the mean levels of Cu, Zn and Se were within the normal reference range. In conclusion, this study gives the first documentation to which extent Danish dairy cattle are exposed to Glyphosate and its impact on blood parameters.

Artigo completo disponível em http://www.omicsonline.org/field-investigations-of-glyphosate-in-urine-of-danish-dairy-cows-2161-0525.1000186.pdf

Krüger, M.; Schledorn, P.; Schrödl, W.; Hoppe, H.; Lutz, W.; Shehata, A. 2014. Detection of glyphosate residues in animals and humans. *Journal Environmental Analytical Toxicology*, 4: 210. doi: 10.4172/2161-0525.1000210.

In the present study glyphosate residues were tested in urine and different organs of dairy cows as well as in urine of hares, rabbits and humans using ELISA and Gas Chromatography-Mass Spectroscopy (GC-MS). The correlation coefficients between ELISA and GC-MS were 0.96, 0.87, 0.97and 0.96 for cattle, human, and rabbit urine and organs, respectively. The recovery rate of glyphosate in spiked meat using ELISA was 91%. Glyphosate excretion in German dairy cows was significantly lower than Danish cows. Cows kept in genetically modified free area had significantly lower glyphosate concentrations in urine than conventional husbandry cows. Also glyphosate was detected in different organs of slaughtered cows as intestine, liver, muscles, spleen and kidney. Fattening rabbits showed significantly higher glyphosate residues in urine than hares. Moreover, glyphosate was significantly higher in urine of humans with conventional feeding. Furthermore, chronically ill humans showed significantly higher glyphosate residues in urine than healthy population. The presence of glyphosate residues in both humans and animals could haul the entire population towards numerous health hazards, studying the impact of glyphosate residues on health is warranted and the global regulations for the use of glyphosate may have to be re-evaluated.

Artigo completo disponível em http://omicsonline.org/open-access/detection-of-glyphosate-residues-in-animals-and-humans-2161-0525.1000210.pdf

Krüger, M.; Schrödl, W.; Pedersen, I.; Shehata, A. 2014. Detection of glyphosate in malformed piglets. *Journal Environmental Analytical Toxicology*, 4: 230. doi: 10.4172/2161-0525.1000230.

Glyphosate residues in different organs and tissues as lungs, liver, kidney, brain, gut wall and heart of malformed euthanized one-day-old Danish piglets (N= 38) were tested using ELISA. All organs or tissues had glyphosate in different concentrations. The highest concentrations were seen in the lungs (Range 0.4-80 µg/ml) and hearts (Range 0.15-80 µg/ml). The lowest concentrations were detected in muscles (4.4-6.4 µg/g). The detection of such glyphosate concentrations in these malformed piglets could be an allusion to the cause of these congenital anomalies. Further investigations are urgently needed to prove or exclude the role of glyphosate in malformations in piglets and other animals.

Artigo completo disponível em http://omicsonline.org/open-access/detection-of-glyphosate-in-malformed-piglets-2161-0525.1000230.pdf

2.1.3 Herbicidas à base de glufosinato de amônio

O glufosinato de amônio é outro princípio ativo de herbicidas associado a plantas transgênicas do tipo TH, fato que assegura sua larga disseminação em vários agroecossistemas. Os artigos a seguir mostram impactos negativos de herbicidas formulados com base naquele princípio ativo sobre organismos humanos e animais, mesmo quando em pequenas doses.

Watanabe, T.; Iwase, T. 1996. Development and dymorphogenic effects of glufosinate ammonium on mouse embryos in culture. *Teratogenesis Carcinogenesis and Mutagenesis*, 16, 287-299.

The effects of glufosinate ammonium on embryonic development in mice were examined using whole embryo and micromass cultures of midbrain and limb bud cells. In day 8 embryos cultured for 48 hr, glufosinate caused significant overall embryonic growth retardation and increased embryolethality to 37.5% at 10 micrograms/ml (5.0 x 10(-5) M). All embryos in the treated groups exhibited specific morphological defects including hypoplasia of the prosencephalon (forebrain) (100%) and visceral arches (100%). In day 10 embryos cultured for 24 hr, glufosinate significantly reduced the crown-rump length and the number of somite pairs, and produced a high incidence of morphological defects (84.6%) at 10 micrograms/ml. These embryos were characterized by blister in the lateral head (100%), hypoplasia of prosencephalon (57.1%), and cleft lips (42.9%) at 20 micrograms/ml (10.0 x 10(-5) M). Histological examination of the treated embryos showed numerous cell death (pyknotic debris) present throughout the neuroepithelium in the brain vesicle and neural tube, but did not involve the underlying mesenchyme. In micromass culture, glufosinate inhibited the differentiation of midbrain cells in day 12 embryos with 50% inhibition occurring at 0.55 microgram/ml (2.8 x 10(-6) M). The ratios of 50% inhibition concentration for cell proliferation to cell differentiation in limb bud cells were 0.76 and 1.52 in day 11 and 12 embryos, respectively. These findings indicate that glufosinate ammonium is embryotoxic in vitro. In addition to causing growth retardation, glufosinate specifically affected the neuroepithelium of the brain vesicle and neural tube, leading to neuroepithelial cell death.

http://www.ncbi.nlm.nih.gov/pubmed/9178451

Fujii, T. 1997. Transgenerational effects of maternal exposure to chemicals on the functional development of the brain in the offspring. *Cancer Causes and Control*, 8, pp. 524-528.

In order to prevent health risk from environmental chemicals, particularly for progeny, we have been performing a risk assessment for various chemicals including therapeutic agents. This paper reports the functional effects of maternal exposure to psychoactive drugs, anticancer drugs, or herbicides on the offspring of rats. Maternal exposure to imipramine in a dose equivalent to the therapeutic dose per unit body weight induced hyperthermic response to chlorpromazine in the male offspring, while normal control rats showed a marked hypothermia. Exposure to ethosuximide resulted in an increase in play fighting behavior in young offspring that was fostered by lactating normal mothers. Single exposures to nimustine or cisplatin, anticancer drugs, at a different gestational stage resulted in an acceleration of growth when exposed at the earlier stage of gestation. Moreover, cisplatin-exposed rats were emotionally unstable, showing a short latent time to the first line-crossing in an open-field during infantile period. The rats exposed to glufosinate ammonium, an herbicide, during the time of neurogenesis in the hippocampus showed a decrease in the wet-dog shakes response to kainic acid at six weeks of age. These results suggest that maternal exposure to chemicals during pregnancy induces a variety of functional abnormalities in the brain of the offspring dependent on the pharmacologic action of chemicals and the stage of gestation even with a single exposure.

http://www.ncbi.nlm.nih.gov/pubmed/9498910

Koyama, K.; Koyama, K.; Goto, K. 1997. Cardiovascular effects of a herbicide containing glufosinate and a surfactant: *In Vitro* and *in Vivo* analyses in rats. *Toxicology and Applied Pharmacology*, 145, 409–414 (1997).

A herbicide, Basta (BASTA), containing glufosinate ammonium (GLA) as the main component and an anionic surfactant, sodium polyoxyethylene alkylether sulfate (AES), causes hemodynamic changes characterized by a decrease in total vascular resistance with an increase or a decrease in cardiac output in human acute oral poisoning. With a motivation based on these clinical observations, we tried to elucidate the exact component and its mode of action that is mostly responsible for the direct cardiovascular effects of this herbicide formulation, investigating the effects of BASTA, GLA, and AES independently on the cardiovascular system in rats in vitro and in vivo. In isolated right atria beating spontaneously in Krebs-Ringer's solution, BASTA and AES produced negative chronotropic responses in a concentration-dependent manner. In electrically driven isolated left atria, BASTA and AES produced positive inotropic responses concentration dependently but negative inotropic responses at extremely high concentrations. In aortic ring segments, BASTA and AES produced no vasoconstrictive effects but exerted significant vasodilative effects when the aortic ring was precontracted with phenylephrine. These in vitro responses caused by BASTA and AES occurred to a similar degree. On the other hand, the main component, GLA, produced no effects in isolated atria and aortas. In anesthetized rats, relatively low doses of BASTA and AES produced a decrease in blood pressure followed by a slight increase in heart rate, which was presumably due to baroreflex caused by the decrease in blood pressure. At an extremely high dose, BASTA and AES produced a decrease in blood pressure with a marked decrease in heart rate. These in vivo responses to BASTA and AES also occurred to a similar degree. In contrast, the main component, GLA, did not produce any effects on heart rate and blood pressure in anesthetized rats. From these results, we concluded that the effects of BASTA in our in vivo experiments were not caused by the main component, GLA, but was mostly caused by AES through its vasodilative effects plus cardiostimulatory effects at low doses and cardiosuppressiveeffects at high doses.

http://www.ncbi.nlm.nih.gov/pubmed/9266815

Matsumura, N.; Takeuchi, C.; Hishikawa, K.; Fujii, T.; Nakaki, T. 2001. Glufosinate ammonium induces convulsion through N-methyl-d-aspartate receptors in mice. *Neuroscience Letters*, 304, 123-125.

Glufosinate ammonium, a broad-spectrum herbicide, causes convulsion in rodents and humans. Because of the structural similarities betweenglufosinate and glutamate, the convulsion induced by glufosinate ammonium may be ascribed to glutamate receptor activation. Three N-methyl-D-asparate (NMDA) receptor antagonists, dizocilpine, LY235959, and Compound 40, and an alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptor antagonist, NBQX, were coadministrated with glufosinate ammonium (80 mg/kg, intraperitoneally) in mice. Statistical analyses showed that the NMDA receptor antagonists markedly inhibited the convulsions, while the AMPA/kainate receptor antagonist had no effect on the convulsion. These results suggest that the convulsion caused by glufosinate ammonium is mediated through NMDA receptors.

http://www.ncbi.nlm.nih.gov/pubmed/11335070

2.1.4 Herbicidas à base de 2,4-D

Atualmente as plantas GM TH incluem em seu portfólio associação com herbicidas formulados com base no 2,4-D. Isso assegura que seu emprego será crescente e ocupará espaços associados ao glifosato e ao glufosinato de amônio, que mostram queda de eficácia no controle de plantas consideradas indesejáveis. Eventos GM com essa característica têm sido recentemente autorizados para plantio comercial no Canadá, nos EUA e no Brasil.

Os estudos a seguir mostram que tal herbicida, além de cancerígeno (Linfoma Não-Hodgkin), provoca efeitos genotóxicos, teratogênicos, perturbações endócrinas e danos nos sistemas reprodutivo e nervoso, nos fígado e rins. Mesmo em pequenas doses, os impactos se revelam importantes em casos de estudos envolvendo organismos de animais modelos e seres humanos.

Outros artigos ainda apontam o fato de herbicidas à base de 2,4-D (em especial as formulações mais baratas, comercializadas irregularmente e com origem pouco clara) conterem contaminantes extremamente perigosos, como a dioxina e outras substâncias altamente cancerígenas.

EPI – Hoar, S.; Blair, A.; Holmes, F.; Boysen, C.; Robel, R.; Hoover, R.; Fraumeni, J. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256:1141-1147.

A population-based case-control study of soft-tissue sarcoma (STS), Hodgkin's disease (HD), and non-Hodgkin's lymphoma (NHL) in Kansas found farm herbicide use to be associated with NHL (odds ratio [OR], 1.6; 95% confidence interval [CI], 0.9, 2.6). Relative risk of NHL increased significantly with number of days of herbicide exposure per year and latency. Men exposed to herbicides more than 20 days per year had a sixfold increased risk of NHL (OR, 6.0; 95% CI, 1.9, 19.5) relative to nonfarmers. Frequent users who mixed or applied the herbicides themselves had an OR of 8.0 (95% CI, 2.3, 27.9) for NHL. Excesses were associated with use of phenoxyacetic acid herbicides, specifically 2,4-dichlorophenoxyacetic acid. Neither STS nor HD was associated with pesticide exposure. This study confirms the reports from Sweden and several US states that NHL is associated with farm herbicide use, especially phenoxyacetic acids. It does not confirm the case-control studies or the cohort studies of pesticide manufacturers and Vietnam veterans linking herbicides to STS or HD.

http://www.ncbi.nlm.nih.gov/pubmed/3801091

EPI – Zahm, S.; Weisenburger, D.; Babbit, P.; Saal, R.; Vaught, J.; Cantor, K.; Blair, A. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in Eastern Nebraska. *Epidemiology*, 1:349–356.

To evaluate the role of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the development of non-Hodgkin's lymphoma (NHL), we conducted a population-based, case-control study in 66 counties in eastern Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July 1, 1983, and June 30, 1986, and with 725 controls. There was a 50% excess of NHL among men who mixed or applied 2,4-D (odds ratio [OR] = 1.5; 95% confidence interval = 0.9, 2.5). The risk of NHL increased with the average frequency of use to over threefold for those exposed 20 or more days per year (p for trend = 0.051). Adjusting for use of organophosphate insecticides lowered the risk estimate for frequent users (OR = 1.8), but adjustment for fungicide use increased the risk estimate (OR = 4.5). Simultaneous adjustment for organophosphates and fungicides yielded an OR of 3.1 for farmers who mixed or applied 2,4-D more than 20 days per year. Risk also increased with degree of exposure, as indicated by application method and time spent in contaminated clothing, but not with the number of years of 2,4-D use or failure to use protective equipment. Although other pesticides, especially organophosphate insecticides, may be related to NHL, the risk associated with 2,4-D does not appear to be explained completely by these other exposures.

http://www.ncbi.nlm.nih.gov/pubmed/2078610

EPI – Lerda, D.; Rizzi, R. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). *Mutat Res Letters*, 262: 47–50.

We studied the reproductive function of 32 male farm sprayers who were exposed to 2,4-D. Sperm analysis was made after 4 days of sexual inactivity. Parameters analyzed were volume, sperm count, mobility and morphology. Exposure level was estimated by measuring the concentration of 2,4-

D in the urine. Significant levels of asthenospermia, necrospermia and teratospermia were found in exposed workers compared with unexposed controls. Over time, asthenospermia and necrospermia diminished but the abnormal spermatozoa (teratospermia) continued.

http://www.ncbi.nlm.nih.gov/pubmed/1986284

EPI – Hayes, H.; Tarone, R.; Cantor, K.; Jessen, C.; McCurnin, D.; Richardson, R. 1991. Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J Natl Cancer Inst*, 83:1226–1231.

A hospital-based case-control study of companion dogs examined the risk of developing canine malignant lymphoma associated with the use of chemicals in and about the home. Information from a self-administered owner questionnaire and/or a telephone interview of about 491 cases, 466 nontumor controls, and 479 tumor controls indicated that owners in households with dogs that developed malignant lymphoma applied 2,4-dichlorophenoxyacetic acid (2,4-D) herbicides to their lawn and/ or employed commercial lawn care companies to treat their yard significantly more frequently than control owners (odds ratio = 1.3). In addition, the risk of canine malignant lymphoma rose to a twofold excess with four or more yearly owner applications of 2,4-D. The findings in this study are consistent with occupational studies in humans, which have reported modest associations between agricultural exposure to 2,4-D and increased risk of non-Hodgkin's lymphoma, the histology and epidemiology of which are similar to those of canine malignant lymphoma. The present study suggests that human health implications of 2,4-D exposure in the home environment should receive further investigation

http://www.ncbi.nlm.nih.gov/pubmed/1870148

EPI – Hardell, L.; Eriksson, M. 1999. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer*, 85:1353-1360.

Background: The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.
Methods: A population-based case-control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Univariate and multivariate analyses were performed with the SAS statistical data program.
Results: Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0-2.5) and fungicides (OR, 3.7; 95% CI, 1.1-13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9-2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0-6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.
Conclusions: Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.

Artigo completo disponível em http://www.beyondpesticides.org/documents/acs-nhlymphoma-1999.pdf

REV – Gandhi, R.; Wandji, S-A.; Snedeker, S. 2000. Critical evaluation of cancer risk from 2,4-D. *Reviews of Environmenta Contamination and Toxicology*, vol. 167, p. 1–33.

2,4-D [(2,4-dichlorophenoxy)-acetic acid] is a widely used postemergent herbicide. It is structurally similar to indoleacetic acid (IAA), a naturally occurring plant hormone (see Fig.1). This similarity allows 2,4-D to mimic IAA and is the basis for its herbicidal action. The synthesis of 2,4-D was first reported in 1941 (ARC 1986I). In 1945, Dow Chemical Co. discovered that a 1:1 mixture of 2,4-D and 2,4,5-T [(2,4,5-trichlorophenoxy)-acetic acid] was a more effective herbicide than either of the two chemicals alone. The mixture was widely used thereafter and referred to as Agent Orange (Lilienfeld and Gallo 1989). Exposure to 2,4-D in the past may have more often been to a mixture of herbicides rather than 2,4-D alone. 2,4-D preparations before 1975 were often contaminated with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) (IARC 1986;Johnson et al. 1992).

http://link.springer.com/chapter/10.1007%2F978-1-4612-1156-3_1

Kobal, S.; Cebulj-Kadunc, N.; Cestnik, V. 2000. Serum T3 and T4 concentrations in the adult rats treated with herbicide 2,4-dichlorophenoxyacetic acid. *Pflugers Archiv European Journal of Physiology*, vol. 440 (7), R171-2.

The influence of sublethal doses of 2,4-dichlorophenoxyacetic acid (2,4-D) on serum T3 and T4 concentrations in Hsd Cpb: Wistar rats of both sexes was studied. The trial was performed on 24 males and females respectively, each divided into three groups of 8 animals (control, groups 1 and 2). Aqueous solution of the compound (11 mg/kg body weight--group 1 and 110 mg/kg body weight--group 2) or clean tap water (control group) was used. Aliquots of 2.4 ml/kg body weight were administered with a stomach tube from the 1st to 10th day of the experiment. Three days before the first treatment and on the 6th and 13th day of the experiment the serum T3 and T4 concentrations were determined by commercial radioimmunoassay kits (Byk-Sangtec Diagnostica), validated for rats. A significant decrease of serum T4 ($P < 0.01$) and T3 ($P < 0.001$) was determined in males of groups 1 and 2 during the experiment. On the 6th day of experiment serum T4 and T3 values were significantly lower ($P < 0.001$ and 0.01 respectively) in group 2 than in the controls and group 1 of both males and females. During the whole experiment serum T4 levels were lower in females than in males ($P < 0.05$)

http://www.ncbi.nlm.nih.gov/pubmed/11005658

Schreinemachers, D. 2000. Cancer mortality in four northern wheat-producing states. *Environmental Health Perspectives*, 108:873–881.

Chlorophenoxy herbicides are used both in cereal grain agriculture and in nonagricultural settings such as right-of-ways, lawns, and parks. Minnesota, North Dakota, South Dakota, and Montana grow most of the spring and durum wheat produced in the United States. More than 90% of spring and durum wheat is treated with chlorophenoxy herbicides, in contrast to treatment of approximately 30% of winter wheat. In this ecologic study I used wheat acreage as a surrogate for exposure to chlorophenoxy herbicides. I investigated the association of chlorophenoxy herbicides with cancer mortality during 1980-1989 for selected counties based on level of agriculture ([greater and equal to] 20%) and rural population ([greater and equal to] 50%). Age-standardized cancer mortality rates were determined for grouped counties based on tertiles of wheat acreage per county or for individual counties for frequently occurring cancers. The cancer sites that showed positive trends of increasing cancer mortality with increasing wheat acreage were esophagus, stomach, rectum, pancreas, larynx, prostate, kidney and ureter, brain, thyroid, bone, and all cancers (men) and oral cavity and tongue, esophagus, stomach, liver and gall bladder and bile ducts, pancreas, cervix, ovary, bladder, and other urinary organs, and all cancers (women). Rare cancers in men and women and cancers in boys and girls were studied by comparing counties above and below the median of wheat acreage per county. There was increased mortality for cancer of the nose and eye in both men and women, brain and leukemia in both boys and girls, and all cancers in boys. These results suggest an association between cancer mortality and wheat acreage in counties of these four states.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2556929/pdf/ehp0108-000873.pdf

Fofana, D.; Kobae, H.; Oku, S.; Nishi, J-I.; Miyata, K. 2000. Prenatal developmental effects of pure 2,4-dichlorophenoxyacetic acid (2,4-D) on the rat. *Congenital Anomalies*, 40:287–296.

2,4-Dichlorophenoxyacetic acid (2,4-D) a plant growth regulator, has been used worldwide as an herbicide. The phenoxyacetic acid herbicides contain both 2,4-D and 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) along with emulsifiers, solvents and contaminants; these have been recognized as teratogen in the rat and mouse. Although the high teratogenicity of phenoxyacetic herbicides has been attributed to the 2,4,5-T and the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the possibility that 2,4-D might play a role has not been clearly ruled out We designed this study to evaluate the effects of pure 2,4-D, and herein describe the precise fetal visceral malformations. We randomly distributed pregnant Wistar rats in to three main groups according to organogenesis during which pure 2,4-D was administered at different doses: l)-over all organogenesis (gestational days 6 to 15), 2)-early organogenesis (gestational days 6 to 10), and 3)-late organogenesis (gestational days 11 to 15). We found that the pure 2,4-D is maternally toxic and has a dose-related embryolethality. The visceral malformations induced in the fetuses included ureteric dilatations and hydronephrosis, as reported, in conjunction with the herbicide forms. In addition, we observed an association with renal and urogenital aplasia, which were observed in the early organogenesis period. We performed a histopatho-logical examination and discussed the mechanism of the pathological processes. We conclude that the pure 2,4-D itself is maternally toxic and embryolethal, and potential inducer of kidney and urogenital malformations in the rat. The types of kidney and urogenital malformations seen indicated that the 2,4-D interferes in the early developmental stage of the urogenital system.

http://onlinelibrary.wiley.com/doi/10.1111/j.1741-4520.2000.tb00927.x/abstract

REV – Bradberry, S.; Watt, B.; Proudfoot, A.; Vale, J. 2000. Mechanisms of toxicity, clinical features, and management of acute chlorophenoxy herbicide poisoning: a review. *J Toxicol Clin Toxicol*, vol. 38 (2), p. 111-22.

Introduction: Chlorophenoxy herbicides are used widely for the control of broad-leaved weeds. They exhibit a variety of mechanisms of toxicity including dose-dependent cell membrane damage, uncoupling of oxidative phosphorylation, and disruption of acetylcoenzyme A metabolism. Between January 1962 and January 1999, 66 cases of chlorophenoxy herbicide poisoning following ingestion were reported in the literature.

Features Following Ingestion: Adjuvants in the formulations may have contributed to some of the features observed. Vomiting, abdominal pain, diarrhea, and, occasionally, gastrointestinal hemorrhage were early effects. When present, hypotension was predominantly due to intravascular volume loss, although vasodilation and direct myocardial toxicity may have contributed in some cases. Neurotoxic features included coma, hypertonia, hyperreflexia, ataxia, nystagmus, miosis, hallucinations, convulsions, fasciculation, and paralysis. Hypoventilation occurred not infrequently, usually in association with central nervous system depression, but respiratory muscle weakness was a factor in the development of respiratory failure in some patients. Myopathic symptoms including limb muscle weakness, loss of tendon reflexes, and myotonia were observed and increased creatine kinase activity was noted in some cases. Other clinical features reported included metabolic acidosis, rhabdomyolysis, renal failure, increased aminotransferase activities, pyrexia, and hyperventilation. Twenty-two of 66 patients died.

Features Following Dermal and Inhalational Exposure: Substantial dermal or inhalational 2,4-dichlorophenoxyacetic acid exposure has occasionally led to systemic features but no such reports have been published in the last 20 years and no fatalities have been reported at any time. Substantial dermal exposure has been reported to cause mild gastrointestinal irritation after a latent period followed by progressive mixed sensory-motor peripheral neuropathy. Mild, transient gastrointestinal and peripheral neuromuscular symptoms have also occurred after occupational inhalation exposure, with or without dermal exposure.

Management: In addition to supportive care, alkaline diuresis to enhance herbicide elimination should be considered in all seriously poisoned patients. Limited clinical data suggest that hemodialysis produces similar herbicide clearance to alkaline diuresis without the need for urine pH manipulation and the administration of substantial amounts of intravenous fluid in an already compromised patient.

Conclusions: While chlorophenoxy herbicide poisoning is uncommon, ingestion of a chlorophenoxy herbicide can result in serious and sometimes fatal sequelae. In severe cases of poisoning, alkaline diuresis or hemodialysis to increase herbicide elimination should be considered.

http://www.ncbi.nlm.nih.gov/pubmed/10778907

EPI – Garry, V.; Tarone, R.; Kirsch, I.; Abdallah, J.; Lombardi, D.; Long, L.; Burroughs, B.; Barr, D.; Kesner, J. 2001. Biomarker correlations of urinary 2,4-D levels in foresters: genomic instability an endocrine disruption. *Environmental Health Perspectives*, vol. 109 (5), p. 495-500.

Forest pesticide applicators constitute a unique pesticide use group. Aerial, mechanical-ground, and focal weed control by application of herbicides, in particular chlorophenoxy herbicides, yield diverse exposure scenarios. In the present work, we analyzed aberrations in G-banded chromosomes, reproductive hormone levels, and polymerase chain reaction-based V(D)J rearrangement frequencies in applicators whose exposures were mostly limited to chlorophenoxy herbicides. Data from appliers where chlorophenoxy use was less frequent were also examined. The biomarker outcome data were compared to urinary levels of 2,4-dichlorophenoxyacetic acid (2,4-D) obtained at the time of maximum 2,4-D use. Further comparisons of outcome data were made to the total volume of herbicides applied during the entire pesticide-use season. Twenty-four applicators and 15 minimally exposed foresters (control) subjects were studied. Categorized by applicator method, men who used a hand-held, backpack sprayer in their applications showed the highest average level (453.6 ppb) of 2,4-D in urine. Serum luteinizing hormone (LH) values were correlated with urinary 2,4-Dlevels, but follicle-stimulating hormone and free and total testosterone were not. At the height of the application season; 6/7 backpack sprayers, 3/4 applicators who used multinozzle mechanical (boom) sprayers, 4/8 aerial applicators, and 2/5 skidder-radiarc (closed cab) appliers had two or more V(D)J region rearrangements per microgram of DNA. Only 5 of 15 minimally exposed (control) foresters had two or more rearrangements, and 3 of these 5 subjects demonstrated detectable levels of 2,4-D in the urine. Only 8/24 DNA samples obtained from the exposed group 10 months or more after their last chlorophenoxy use had two rearrangements per microgram of DNA, suggesting that the exposure-related effects observed were reversible and temporary. Although urinary 2,4-D levels were not correlated with chromosome aberration frequency, chromosome aberration frequencies were correlated with the total volume of herbicides applied, including products other than 2,4-D. In summary, herbicide applicators with high urinary levels of 2,4-D (backpack and boom spray applications) exhibited elevated LH levels. They also exhibited altered genomic stability as measured by V(D)J rearrangement frequency, which appears reversible months after peak exposure. Though highly detailed, the limited sample size warrants cautious interpretation of the data.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1240309/

EPI – Arbuckle, T.; Lin, Z.; Mery, L. 2001. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. *Environ Health Perspect*, 109:851–857.

The toxicity of pesticides on human reproduction is largely unknown--particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12-19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1-2.1], triazines (OR = 1.4; 95% CI, 1.0-2.0), and any herbicide (OR = 1.4; 95% CI, 1.1-1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0-2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1-3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0-2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1240415/pdf/ehp0109-000851.pdf

Amer, S.; Aly, F. 2001. Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse. *Mutat Res*, 494(1-2):1-12.

The cytogenetic effect of 2,4-dichlorophenoxy acetic acid (2,4-D) and its metabolite 2,4-dichlorophenol (2,4-DCP) was studied in bone-marrow, germ cells and sperm head abnormalities in the treated mice. Swiss mice were treated orally by gavage with 2,4-D at 1.7, 3.3 and 33 mg kg(-1)BW (1/200, 1/100 and 1/10 of LD(50)). 2,4-DCP was intraperitoneally (i.p.) injected at 36, 72 and 180 mg kg(-1)BW (1/10, 1/5, 1/2 of LD(50)). A significant increase in the percentage of chromosome aberrations in bone-marrow and spermatocyte cells was observed after oral administration of 2,4-D at 3.3 mg kg(-1)BW for three and five consecutive days. This percentage increased and reached 10.8+/-0.87 (P<0.01) in bone-marrow and 9.8+/-0.45 (P<0.01) in spermatocyte cells after oral administration of 2,4-D at 33 mg kg(-1)BW for 24 h. This percentage was, however, lower than that induced in bone-marrow and spermatocyte cells by mitomycin C (positive control). 2,4-D induced a dose-dependent increase in the percentage of sperm head abnormalities. The genotoxic effect of 2,4-DCP is weaker than that of 2,4-D, as indicated by the lower percentage of the induced chromosome aberrations (in bone-marrow and spermatocyte cells) and sperm head abnormalities. Only the highest tested concentration of 2,4-DCP (180 mg kg(-1)BW, 1/2 LD(50)) induced a significant percentage of chromosome aberrations and sperm head abnormalities after i.p. injection. The obtained results indicate that 2,4-D is genotoxic in mice in vivo under the conditions tested. Hence, more care should be given to the application of 2,4-D on edible crops since repeated uses may underlie a health hazard.

http://www.ncbi.nlm.nih.gov/pubmed/11423340

Holland, N.; Duramad, P.; Rothman, N.; Figgs, L.; Blair, A.; Hubbard, A.; Smith, M. 2002. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid *in vitro* and *in vivo*. *Mutat Res*, 26;521(1-2):165-78.

Widespread use of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its association with non-Hodgkin's lymphoma (NHL) and other cancers has raised public concern. Here, micronucleus (MN) formation has been used as a biomarker of genotoxicity, and replicative and mitotic indices (MIs) as biomarkers of cell cycle kinetics in human lymphocytes. Cells were cultured either as whole blood or isolated lymphocytes and treated with pure or commercial forms of 2,4-D at doses between 0.001 and 1 mM for 48 h. Exposure to 2,4-D produced a minimal increase in MN in whole blood and even smaller one in isolated lymphocyte cultures. This induction took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). At a low (0.005 mM) dose of commercial 2,4-D, a small, marginally significant increase in RI (12-15%) was found in two independent sets of experiments (P=0.052). Additionally, we found that lymphocyte RI was more affected by commercial 2,4-D containing 9.4% of the chemically pure 2,4-D, than with an equal concentration of the latter suggesting that other ingredients present in the commercial pesticide may be responsible or may enhance the effect of 2,4-D. Mitotic index, however, did not show any significant change with either commercial or pure 2,4-D. The lymphocytes of 12 male applicators exposed solely to 2,4-D during a 3-month period had a significantly higher RI than the same group prior to exposure and than a control group (P<0.01), in accordance with the in vitro finding of increased RI at low doses.

http://www.ncbi.nlm.nih.gov/pubmed/12438013

Bukowska, B. 2003. Effects of 2,4-D and its metabolite 2,4-dichlorophenol on antioxidant enzymes and level of glutathione in human erythrocytes. *Comp Biochem Physiol C Toxicol Pharmacol*, 135(4): 435-41.

The effects of in vitro exposure of human erythrocytes to different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and its metabolite 2,4-dichlorophenol (2,4-DCP) were studied. The activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and the level of reduced glutathione (GSH) were determined. The activity of erythrocyte superoxide dismutase SOD decreased with increasing dose of 2,4-D and 2,4-DCP, while glutathione peroxidase activity increased. 2,4-D (500 ppm) decreased the level of reduced glutathione in erythrocytes by 18% and 2,4-DCP (250 ppm) by 32%, respectively, in comparison with the controls. These results lead to the conclusion that in vitro administration of herbicide-2,4-D and its metabolite 2,4-DCP causes a decrease in the level of reduced glutathione in erythrocytes and significant changes in antioxidant enzyme activities. Comparison of the toxicity of 2,4-D and 2,4-DCP revealed that the most prominent changes occurred in human erythrocytes incubated with 2,4-DCP.

http://www.ncbi.nlm.nih.gov/pubmed/12965188

Schreinemachers, D. 2003. Birth malformations and other adverse perinatal outcomes in four US wheat-producing states. *Env Health Perspec*, 111(9) 1259-1264.

Chlorophenoxy herbicides are widely used in the United States and Western Europe for broadleaf weed control in grain farming and park maintenance. Most of the spring and durum wheat produced in the United States is grown in Minnesota, Montana, North Dakota, and South Dakota, with more than 85% of the acreage treated with chlorophenoxy herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA). Rates of adverse birth outcomes in rural, agricultural counties of these states during 1995-1997 were studied by comparing counties with a high proportion of wheat acreage and those with a lower proportion. Information routinely collected and made available by federal agencies was used for this ecologic study. Significant increases in birth malformations were observed for the circulatory/respiratory category for combined sexes [odds ratio (OR) = 1.65; 95% confidence interval (CI), 1.07-2.55]. A stronger effect was observed for the subcategory, which excluded heart malformations (OR = 2.03; 95% CI, 1.14-3.59). In addition, infants conceived during April-June--the time of herbicide application--had an increased chance of being diagnosed with circulatory/respiratory (excluding heart) malformations compared with births conceived during other months of the year (OR = 1.75; 95% CI, 1.09-2.80). Musculoskeletal/integumental anomalies increased for combined sexes in the high-wheat counties (OR = 1.50; 95% CI, 1.06-2.12). Infant death from congenital anomalies significantly increased in high-wheat counties for males (OR = 2.66; 95% CI, 1.52-4.65) but not for females (OR = 0.48; 95% CI, 0.20-1.15). These results are especially of concern because of widespread use of chlorophenoxy herbicides.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241584/pdf/ehp0111-001259.pdf

REV – Bradberry, S.; Proudfoot, A.; Vale, J. 2004. Poisoning due to chlorophenoxy herbicides. *Toxicol Rev*, vol. 23 (2), p. 65-73.

Chlorophenoxy herbicides are used widely for the control of broad-leaved weeds. They exhibit a variety of mechanisms of toxicity including dose-dependent cell membrane damage, uncoupling of oxidative phosphorylation and disruption of acetylcoenzyme A metabolism. Following ingestion, vomiting, abdominal pain, diarrhoea and, occasionally, gastrointestinal haemorrhage are early effects. Hypotension, which is common, is due predominantly to intravascular volume loss, although vasodilation and direct myocardial toxicity may also contribute. Coma, hypertonia, hyperreflexia, ataxia, nystagmus, miosis, hallucinations, convulsions, fasciculation and paralysis may then ensue. Hypoventilation is commonly secondary to CNS depression, but respiratory muscle weakness is a factor in the development of respiratory failure in some patients. Myopathic symptoms including limb muscle weakness, loss of tendon reflexes, myotonia and increased creatine kinase activity have been observed. Metabolic acidosis, rhabdomyolysis, renal failure, increased aminotransferase activities, pyrexia and hyperventilation have been reported. Substantial dermal exposure to 2,4-dichlorophenoxy acetic acid (2,4-D) has led occasionally to systemic features including mild gastrointestinal irritation and progressive mixed sensorimotor peripheral neuropathy. Mild, transient gastrointestinal and peripheral neuromuscular symptoms have occurred after occupational inhalation exposure. In addition to supportive care, urine alkalinization with high-flow urine output will enhance herbicide elimination and should be considered in all seriously poisoned patients. Haemodialysis produces similar herbicide clearances to urine alkalinization without the need for urine pH manipulation and the administration of substantial amounts of intravenous fluid in an already compromised patient.

http://www.ncbi.nlm.nih.gov/pubmed/15578861

Sameshima, K.; Kobae, H.; Fofana, D.; Yoshidome, K.; Nishi, J.; Miyata, K. 2004. Effects of pure 2,4-dichlorophenoxyacetic acid on cultured rat embryos. *Congenit Anom (Kyoto)*, 44(2):93-6.

2,4-dichlorophenoxyacetic acid (2,4-D), a plant growth regulator, has been used worldwide as a herbicide. Previously we evaluated the prenataldevelopmental effects of 2,4-D by feeding it to pregnant rats and found that it is maternally toxic and embryolethal, and it induces urogenital malformations in rat fetuses. In the study presented here, we investigated the effects of pure 2,4-D on rat embryos in whole embryo culture. Ratembryos on day 9.5 of gestation were cultured for 48 h at several concentration levels with pure 2,4-D (50-500 microg/mL). 2,4-D caused a concentration-related increase in the incidence of each malformation. Significant decreases in the number of somites were observed at a concentration of 100 microg/mL or more. At the concentration of 100 microg/mL, there was normal yolk sac circulation. This result suggests that 2,4-D has a detrimental effect on somite development and directly damages developing embryos.

http://www.ncbi.nlm.nih.gov/pubmed/15198722

Bortolozzi, A.; Duffard, A.; Duffard, R.; Antonelli, M. 2004. Effects of 2,4-dichlorophenoxyacetic acid exposure on dopamine D2-like receptors in rat brain. *Neurotoxicol Teratol*, 26(4):599-605.

2,4-Dichlorophenoxyacetic acid (2,4-D), a worldwide-used herbicide, has been associated with a range of adverse health effects on humans and different animal species. Although the mechanism of 2,4-D neurotoxicity remains unknown, we had previously reported changes in various neurotransmitter systems, such as serotonin (5-HT) and dopamine (DA), which were proposed to mediate some of the behavioral effects in rats. In the present work, we examined the impact of 2,4-D exposure on the ontogeny of dopaminergic D2-type receptors in prefrontal cortex (PFc), striatum (CPu), hippocampus (H) and cerebellum (Cer). Pregnant rats were orally exposed to 70 mg/kg/day of 2,4-D from gestation day (GD) 16 to postpartum day 23. After weaning, the pups were assigned to one of the two subgroups: T1 [fed with untreated diet until postnatal day, (PD) 90] and T2 [maintained with 2,4-D diet until PD 90]. Five to eight pups per age and sex were sacrificed at 6, 15, 30, 45 or 90 days of age for membrane receptor binding assays employing [3H] nemonapride. Subchronic 2,4-D exposure (T2 group) increased DA D2-type receptor around 40% in CPu. In addition, DA D2-type receptor levels also increased in PFc (15 and 30 days) and Cer (30 and 90 days). Sex-dependent differences in D2 receptors were observed with T2 female rats being more affected than T2 male rats. When the herbicide treatment was interrupted after weaning (T1 group), DA D2-type receptor density was apparently recovered and stabilized to control level. These findings suggest a reversible vulnerability of D2-type receptors to 2,4-D exposure. Regional increases of D2-type receptor density may explain certain behaviors reported early by us, such as catalepsy and right-turning preference in rats exposed to 2,4-D.

http://www.ncbi.nlm.nih.gov/pubmed/15203183

Bharadwaj, L.; Dhami, K.; Stevens, M.; Renaud, C.; Ali, A. 2005. Altered gene expression in human hepatoma HepG2 cells exposed to low-level 2,4-dichlorophenoxyacetic acid and potassium nitrate. *Toxicol In Vitro*, vol. 19 (5), p. 603-19.

2,4-dichlorophenoxyacetic acid (2,4-D) and nitrate are agricultural contaminants found in rural ground water. It is not known whether levels found in groundwater pose a human or environmental health risk, nor is the mechanism of toxicity at the molecular/cellular level understood. This study focused on determining whether 2,4-D or nitrate at environmentally realistic levels elicit gene expression changes in exposed cells. cDNA microarray technology was used to determine the impact of 2,4-D and nitrate in an in vitro model of exposure. Human hepatoma HepG2 cells were incubated with 2,4-D or nitrate alone for 24 h. Cell viability (neutral red assay) and proliferation (BrdU incorporation) were assessed following exposure. Total RNA from treated and control cells were isolated, reverse transcribed and reciprocal labelled with Cy3 or Cy5 dyes, and hybridized to a human cDNA microarray. The hybridized microarray chips were scanned, quantified and analyzed to identify genes affected by 2,4-D or nitrate exposure based on a two-fold increase or decrease in gene expression and reproducibility (affected in three or more treatments). Following filtering, normalization and hierarchical clustering initial data indicate that numerous genes were found to be commonly expressed in at least three or more treatments of 2,4-D or nitrate tested. The affected genes indicate that HepG2 cells respond to environmental, low-level exposure and produce a cellular response that is associated with alterations in the expression of many genes. The affected genes were characterized as stress response, cell cycle control, immunological and DNA repair genes. These findings serve to highlight new pathway(s) in which to further probe the effects of environmental levels of 2,4-D and nitrate.

http://www.ncbi.nlm.nih.gov/pubmed/15878651

González, M.; Soloneski, S.; Reigosa, M.; Larramendy, M. 2005. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic and a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt. I. Evaluation of DNA damage and cytogenetic endpoints in Chinese Hamster ovary (CHO) cells. *Toxicol In Vitro*, 19(2): 289-97.

Genotoxicity of the 2,4-dichlorophenoxyacetic acid (2,4-D) and a commercially-used derivative, 2,4-D dimethylamine salt (2,4-D DMA), was evaluated in CHO cells using SCE and single cell gel electrophoresis (SCGE) assays. Log-phase cells were treated with 2.0-10.0 microg/ml of herbicides and harvested 24 and 36 h later for SCE analysis. Both agents induced significant dose-dependent increases in SCE, regardless of the harvesting time (2,4-D: $r=0.98$ and $r=0.88$, $P<0.01$, for 24 and 36 h harvesting times; 2,4-D DMA: $r=0.97$ and $r=0.88$, $P<0.01$, for 24 and 36 h harvesting times). Neither test compound altered cell-cycle progression or proliferative replication index ($P>0.05$), but the higher doses of both compounds reduced the mitotic index of cultures harvested at 24 and 36 h ($P<0.05$). A 90-min treatment with 2.0-10.0 microg/ml 2,4-D and 2,4-D DMA produced dose-dependent increases in the frequency of DNA-strand breaks detected in the SCGE assay, both in cultures harvested immediately after treatment and in cultures harvested 36 h later. The doses of 2,4-D and 2,4-D DMA were equally genotoxic in all of the assays. The results indicate that 2,4-D induces SCE and DNA damage in mammalian cells, and should be considered as potentially hazardous to humans.

http://www.ncbi.nlm.nih.gov/pubmed/15649642

LaChapelle, A.; Ruygrok, M.; Toomer, M.; Oost, J.; Monnie, M.; Swenson, J.; Compton, A.; Stebbins-Boaz, B. 2007. The hormonal herbicide, 2,4-dichlorophenoxyacetic acid, inhibits *Xenopus oocyte* maturation by targeting translational and posttranslational mechanisms. *Reproductive toxicology*, 23(1):20-31.

The widely used hormonal herbicide, 2,4-dichlorophenoxyacetic acid, blocks meiotic maturation in vitro and is thus a potential environmental endocrine disruptor with early reproductive effects. To test whether maturation inhibition was dependent on protein kinase A, an endogenous maturation inhibitor, oocytes were microinjected with PKI, a specific PKA inhibitor, and exposed to 2,4-D. Oocytes failed to mature, suggesting that 2,4-D is not dependent on PKA activity and likely acts on a downstream target, such as Mos. De novo synthesis of Mos, which is triggered by mRNA poly(A) elongation, was examined. Oocytes were microinjected with radiolabelled in vitro transcripts of Mos RNA and exposed to progesterone and 2,4-D. RNA analysis showed progesterone-induced polyadenylation as expected but none with 2,4-D. 2,4-D-activated MAPK was determined to be cytoplasmic in localization studies but poorly induced Rsk2 phosphorylation and activation. In addition to inhibition of the G2/M transition, 2,4-D caused abrupt reduction of H1 kinase activity in MII phase oocytes. Attempts to rescue maturation in oocytes transiently exposed to 2,4-D failed, suggesting that 2,4-D induces irreversible dysfunction of the meiotic signaling mechanism.

http://www.ncbi.nlm.nih.gov/pubmed/17055699

Arias, E. 2007. Cytogenetic effects of short- and long-term exposure of chick embryos to the phenoxyherbicide 2,4-D. *Environ Mol Mutagen*, 48(6):462-6.

Before incubation, chick embryos were treated with the herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) by injecting onto the inner shell membrane solutions of 0, 0.5, 1, 2, or 4 mg 2,4-D. A commercial formulation containing 37% 2,4-D iso-octyl ester as active ingredient and pure 2,4-D were tested. Sister chromatid exchange (SCE) and cell cycle kinetics were examined at days 4, 7, and 10 from 22 to 30 embryos per group. After 4 days of exposure to commercial 2,4-D, a small ($P < 0.05$) dose-related increase of SCE was seen for the 4-mg group. An enhanced SCE response upon long-term exposure to 2,4-D was apparent. After 10 days of exposure, SCE frequencies for the 2- and 4-mg commercial 2,4-D, and 4-mg pure 2,4-D groups were significantly higher than for the controls. A significant slowing of cell cycle at concentrations at and above 1 mg was seen. Also observed was a slight, not statistically significant proliferative effect at the lowest dose of 0.5 mg/embryo. Consistent with the results from other test systems, the present findings indicate that 2,4-D has a low to moderate genotoxic activity.

http://www.ncbi.nlm.nih.gov/pubmed/17372986

Stürtz, N.; Deis, R.; Jahn, G.; Duffard, R.; Duffard, A. 2008. Effect of 2,4-dichlorophenoxyacetic acid on rat maternal behaviour. *Toxicology*, 247(2-3): 73-79.

Exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) has several deleterious effects on the nervous system such as alterations in the concentrations of neurotransmitters in the brain and/or behavioral changes, myelination rate, ganglioside pattern [Bortolozzi, A., Duffard, R., Antonelli, M., Evangelista de Duffard, A.M., 2002. Increased sensitivity in dopamine D(2)-like brain receptors from 2,4-dichlorophenoxyacetic acid (2,4-D)-exposed and amphetamine-challenged rats. Ann. N.Y. Acad. Sci. 965, 314-323; Duffard, R., García, G., Rosso, S., Bortolozzi, A., Madariaga, M., DiPaolo, O., Evangelista de Duffard, A.M., 1996. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol. Teratol. 18, 691-696; Evangelista de Duffard, A.M., Orta, C., Duffard, R., 1990. Behavioral changes in rats fed a diet containing 2,4-dichlorophenoxyacetic butyl ester. Neurotoxicology 11, 563-572; Evangelista de Duffard, A.M., Bortolozzi, A., Duffard, R.O., 1995. Altered behavioral responses in 2,4-dichlorophenoxyacetic acid treated and amphetamine challenged rats. Neurotoxicology 16, 479-488; Munro, I.C., Carlo, G.L., Orr, J.C., Sund, K., Wilson, R.M. Kennepohl, E. Lynch, B., Jablinske, M., Lee, N., 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. J. Am. Coll. Toxicol. 11, 559-664; Rosso et al., 2000], and its administration to pregnant and lactating rats adversely affects litter growth and milk quality. Since normal growth of the offspring depends on adequate maternal nursing and care, we evaluated the effect of 2,4-D on rat maternal behavior as well as the dam's monoamine levels in arcuate nucleus (AcN) and serum prolactin (PRL) levels. Wistar dams were exposed to the herbicide through the food from post partum day (PPD) 1 to PPD 7. Dams were fed either with a 2,4-D treated diet (15, 25 or 50mg 2,4-D/kg/daybw) or with a control diet. We observed that maternal nesting behavior was not modified by 2,4-D treatment. However, mother-pup interactions, specially the nursing behavior, were altered. Retrieval, crouching and licking of pups were reduced or suspended after 2,4-D treatment. We also observed an increase in the latency of retrieval and crouching in the dams treated with the herbicide. Dams showed movement along cage peripheries, food consumption during the light phase and high self-grooming. In addition of the deficits observed in maternal behavior parameters, increased catecholamine levels and a drastic decrease in indolamine levels in the AcN of treated dams were determined. Serum PRL levels were also diminished by 62%, 68% and 70% with respect to control dams in the 15, 25 and 50mg 2,4-D/kgbw treated dams, respectively. In conclusion, exposure to 2,4-D during the first post partum days produced changes in maternal behavior, serum prolactin and monoamine levels in the AcN of treated dams.

http://www.ncbi.nlm.nih.gov/pubmed/18420331

Maillet, E.; Margolskee, R.; Mosinger, B. 2009. Phenoxy herbicides and fibrates potently inhibit the human chemosensory receptor subunit T1R3. *Journal of Medicinal Chemistry*, 52, 6931–6935 6931. DOI: 10.1021/jm900823s.

We show that phenoxyauxin herbicides and lipid-lowering fibrates inhibit human but not rodent T1R3. T1R3 as a coreceptor in taste cells responds to sweet compounds and amino acids; in endocrine cells of gut and pancreas T1R3 contributes to glucose sensing. Thus, certain effects of fibrates in treating hyperlipidemia and type II diabetes may be via actions on T1R3. Likewise, phenoxy herbicides may have adverse metabolic effects in humans that would have gone undetected in studies on rodents.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2783803/

Stürtz, N.; Jahn, G.; Deis, R.; Rettori, V.; Duffard, R.; Duffard, A. 2010. Effect of 2,4-dichlorophenoxyacetic acid on milk transfer to the litter and prolactin release in lactating rats. *Toxicology*, Volume 271, Issues 1–2, 30, Pages 13-20.

The effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on brain monoamines and the serum level of hormones involved in milk synthesis and on the milk ejection reflex in rats were evaluated. Dams were treated with 2.5, 5, 15, 25, 50 or 70mg 2,4-D/kg bw according to two experimental designs: (a) through food from post partum day 1 (PPD 1) to PPD 16 and the respective control groups or (b) an unique i.p. injection on PPD 11. To measure milk ejection, the litter was separated from the mother at the 11th day of lactation during 8h, returned to their mothers and allowed to suckle for a period of 15min. The procedure was repeated on 3 consecutive days until the end of treatment. The change in litter weight during the suckling period was taken as a measure of the amount of milk ejected during this period. The dams' serum prolactin (PRL), oxytocin (OT) and growth hormone levels were determined by radioimmunoassay. Both treatment regimens produced a dose-dependent decrease in the amount of milk ejected and circulating PRL and OT secreted in response to the suckling stimulus. Administration of OT before returning the pups restored the milk ejection, indicating no impairment in the capacity of the mammary gland to produce and secrete milk. In addition, dopamine levels were increased by the 2,4-D treatments in arcuate nucleus (ArN) and anterior lobe of pituitary gland (AL), while serotonin level was drastically decreased in ArN. 2,4-D treatment increased both calcium-dependent and calcium-independent nitric oxide synthase (NOS) activities in ArN. These results suggest that 2,4-D inhibits the suckling-induced hormone release, milk transfer to the litter at the central level, through a stimulation of hypothalamic NOS and dopamine and by an inhibition of hypothalamic serotonin transmission.

http://www.ncbi.nlm.nih.gov/pubmed/20122984

Outra característica danosa desses herbicidas está em sua rápida e fácil dispersão, agravada pela capacidade de suspensão e deriva. Isso amplia – em abrangência – o público exposto, afetando especialmente populações residentes em comunidades rurais e periurbanas de regiões onde a tecnologia é adotada.

Rudel, R.; Camann, D.; Spengler, J.; Korn, L.; Brody, J. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environmental Science & Technology*, 37(20): 4543-4553. doi:10.1021/es0264596.

Chemicals identified as endocrine-disrupting compounds (EDCs) have widespread consumer uses, yet little is known about indoor exposure. We sampled indoor air and dust in 120 homes, analyzing for 89 organic chemicals identified as EDCs. Fifty-two compounds were detected in air and 66 were detected in dust. These are the first reported measures in residential environments for over 30 of the compounds, including several detected at the highest concentrations. The number of compounds detected per home ranged from 13 to 28 in air and from 6 to 42 in dust. The most abundant compounds in air included phthalates (plasticizers, emulsifiers), o-phenylphenol (disinfectant), 4-nonylphenol (detergent metabolite), and 4-tert-butylphenol (adhesive) with typical concentrations in the range of 50-1500 ng/m3. The penta- and tetrabrominated diphenyl ethers (flame retardants) were frequently detected in dust, and 2,3-dibromo-1-propanol, the carcinogenic intermediate of a flame retardant banned in 1977, was detected in air and dust. Twenty-three pesticides were detected in air and 27 were detected in dust, the most abundant being permethrins and the synergist piperonyl butoxide. The banned pesticides heptachlor, chlordane, methoxychlor, and DDT were also frequently detected, suggesting limited indoor degradation. Detected concentrations exceeded government health-based guidelines for 15 compounds, but no guidelines are available for 28 compounds, and existing guidelines do not consider endocrine effects. This study provides a basis for prioritizing toxicology and exposure research for individual EDCs and mixtures and provides new tools for exposure assessment in health studies.

http://www.ncbi.nlm.nih.gov/pubmed/14594359

EPI - Morgan, M.; Sheldon, L.; Thomas, K.; Egeghy, P.; Croghan, C.; Jones, P.; Chuang, J.; Wilson, N. 2008. Adult and children's exposure to 2,4-D from multiple sources and pathways. *Journal of Exposure Science and Environmental Epidemiology*, 18, 486–494.

In this study, we investigated the 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide exposures of 135 preschool-aged children and their adult caregivers at 135 homes in North Carolina (NC) and Ohio (OH). Participants were randomly recruited from six NC and six OH counties. Monitoring was performed over a 48-h period at the participants' homes. Environmental samples included soil, outdoor air, indoor air, and carpet dust. Personal samples collected by the adult caregivers concerning themselves and their children consisted of solid food, liquid food, hand wipe, and spot urine samples. All samples were analyzed for 2,4-D (free acid form) by gas chromatography/mass spectrometry. 2,4-D was detected in all types of environmental samples but most often in carpet dust samples, with detection frequencies of 83% and 98% in NC and OH, respectively. The median level of 2,4-D in the carpet dust samples was about three times higher in OH homes compared to NC homes (156 vs. 47.5 ng/g, $P<0.0002$). For personal samples, 2,4-D was more frequently detected in the hand wipe samples from OH participants (>48%) than from NC participants (<9%). Hand wipe levels at the 95th percentile were about five times higher for OH children (0.1 ng/cm(2)) and adults (0.03 ng/cm(2)) than for the NC children (0.02 ng/cm(2)) and adults (<0.005 ng/cm(2)). 2,4-D was detected in more than 85% of the child and adult urine samples in both states. The median urinary2,4-D concentration was more than twice as high for OH children compared to NC children (1.2 vs. 0.5 ng/ml, $P<0.0001$); however, the median concentration was identical at 0.7 ng/ml for both NC and OH adults. The intraclass correlation coefficient of reliability for an individual's urinary 2,4-Dmeasurements, estimated from the unadjusted (0.31-0.62) and specific gravity-adjusted (0.37-0.73) values, were somewhat low for each group in this study. The variability in urinary 2,4-D measurements over the 48-h period for both children and adults in NC and OH suggests that several spot samples were needed to adequately assess these participants' exposures to 2,4-D in residential settings. Results from this study showed that children and their adult caregivers in NC and OH were likely exposed to 2,4-D through several pathways at their homes. In addition, our findings suggest that the OH children might have been exposed to higher levels of 2,4-D through the dermal and nondietary routes of exposure than the NC children and the NC and OH adults.

http://www.ncbi.nlm.nih.gov/pubmed/18167507

2.2 Perturbações metabólicas diversas e/ou aspectos de toxicidade relacionados ao consumo de plantas TH (em comparação a controles não GM)

Em que pese o cultivo de fato de PGMs TH ocorra há mais de 15 anos, a comunidade científica se mantém dividida em relação aos riscos que sua presença pode trazer para a saúde e o meio ambiente. As preocupações são mais relevantes quando se consideram possíveis impactos de longo prazo.

Como mencionado no item anterior, parte significativa desses riscos pode decorrer de superposições e sinergias envolvendo o consumo das plantas, os resíduos de caldas herbicidas e os seus principais produtos de degradação.

Ademais, não podem ser descartados efeitos tóxicos ligados a perturbações genéticas, epigenéticas e/ou metabólicas eventualmente presentes nas PGMs. Incertezas e aleatoriedades relacionadas ao processo de transgenia – já discutidos na Parte 1 – não devem ser desconsideradas em verificações de possíveis impactos sobre a saúde dos consumidores.

2.2.1 Soja tolerante aos herbicidas à base de glifosato

A soja RR, tolerante aos herbicidas à base de glifosato, se constitui provavelmente no mais estudado dos eventos transgênicos. A comunidade científica disponibilizou, nos últimos anos, vários artigos apontando riscos toxicológicos para a saúde humana e animal, associados ao seu consumo (são dezenas os estudos publicados que podem ser relacionados aos impactos para a saúde do pacote tecnológico RR). Já outros[58] estudos, mesmo observando diferenças estatisticamente significativas, tendem a interpretá-las como biologi-

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[58] Tais como Zhu et al., 2004 (Nutritional assessment and fate of DNA of soybean meal from roundup ready or conventional soybeans using rats. *Arch Anim Nutr.* 2004, 58:295-310); Tudisco et al., 2006 (Genetically modified soya bean in rabbit feeding: Detection of DNA fragments and evaluation of metabolic effects by enzymatic analysis. *Animal Science*. 82, 193-199); Tudisco et al., 2010 (Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. *Animal.* The Animal Consortium: 1-10; 4:1662-1671).

camente irrelevantes para, a seguir, concluir pela ausência de riscos.

Essa divergência está no centro das controvérsias científicas, sendo atribuída a comprometimentos, metodologias, protocolos e formas de leitura dos dados. Independente das razões, em ambos os casos a precaução recomendaria novos estudos como medida necessária ao estabelecimento de conclusões relativas à biossegurança dessas plantas transgênicas.

Malatesta, M.; Caporaloni, C.; Gavaudan, S.; Rocchi, M.; Serafini, S.; Tiberi, C.; Gazzanelli, G. 2002. Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean. *Cell Struct Funct*, 27, 173180.

No direct evidence that genetically modified (GM) food may represent a possible danger for health has been reported so far; however, the scientific literature in this field is still quite poor. Therefore, we carried out an ultrastructural morphometrical and immunocytochemical study on hepatocytes from mice fed on GM soybean, in order to investigate eventual modifications of nuclear components of these cells involved in multiple metabolic pathways related to food processing. Our observations demonstrate significant modifications of some nuclear features in GM-fed mice. In particular, GM fed-mice show irregularly shaped nuclei, which generally represents an index of high metabolic rate, and a higher number of nuclear pores, suggestive of intense molecular trafficking. Moreover, the roundish nucleoli of control animals change in more irregular nucleoli with numerous small fibrillar centres and abundant dense fibrillar component in GM-fed mice, modifications typical of increased metabolic rate. Accordingly, nucleoplasmic (snRNPs and SC-35) and nucleolar (fibrillarin) splicing factors are more abundant in hepatocyte nuclei of GM-fed than in control mice. In conclusion, our data suggest that GM soybean intake can influence hepatocyte nuclear features in young and adult mice; however, the mechanisms responsible for such alterations remain unknown.

http://www.ncbi.nlm.nih.gov/pubmed/12441651

Malatesta, M.; Caporaloni, C.; Rossi, L.; Battistelli, S.; Rocchi, M.; Tonucci, F.; Gazzanelli, G. 2002. Ultrastructural analysis of pancreatic acinar cells from mice fed on genetically modified soybean. *Journal of Anatomy*, 201:409-415.

No direct evidence that genetically modified (GM) food may represent a possible danger for health has been reported so far; however, the scientific literature in this field is quite poor.Therefore, we investigated the possible effects of a diet containing GM soybean on mouse exocrine pancreas by means of ultrastructural, morphometrical and immunocytochemical analyses. Our observations demonstrate that, although no structural modification occurs in pancreaticacinar cells of mice fed on GM soybean, quantitative changes of some cellular constituents takeplace in comparison to control animals. In particular, a diet containing significant amount of GM food seems to influence the zymogen synthesis and processing.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1570979/

Malatesta, M.; Biggiogera, M.; Manuali, E.; Rocchi, M.; Baldelli, B.; Gazzanelli, G. 2003. Fine structural analysis of pancreatic acinar cell nuclei from mice fed on GM soybean. *Eur J Histochem*, 47, 3858.

We carried out ultrastructural morphometrical and immunocytochemical analyses on pancreatic acinar cell nuclei from mice fed on genetically modified (GM) soybean, in order to investigate possible structural and molecular modifications of nucleoplasmic and nucleolar constituents. We found a significant lowering of nucleoplasmic and nucleolar splicing factors as well as a perichromatin granule accumulation in GM-fed mice, suggestive of reduced post-transcriptional hnRNA processing and/or nuclear export. This is in accordance to already described zymogen synthesis and processing modifications in the same animals.

http://www.ncbi.nlm.nih.gov/pubmed/14706936

Vecchio, L.; Cisterna, B.; Malatesta, M.; Martin, T.; Biggiogera, M. 2004. Ultrastructural analysis of testes from mice fed on genetically modified soybean. *Eur J Histochem*, 48: 448 54.

We have considered the possible effects of a diet containing genetically modified (GM) soybean on mouse testis. This organ, in fact, is a well known bioindicator and it has already been utilized, for instance, to monitor pollution by heavy metals. In this preliminary study, we have focussed our attention on Sertoli cells, spermatogonia and spermatocytes by means of immunoelectron microscopy. Our results point out that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II is decreased in 2 and 5 month-old GM-fed mice, and is restored to normal at 8 months. In GM-fed mice of all ages considered, the number of perichromatin granules is higher and the nuclear pore density lower. Moreover, we found enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells. A possible role played by traces of the herbicide to which the soybean is resistant is discussed.

http://www.ncbi.nlm.nih.gov/pubmed/15718213

Cisterna, B.; Flach, F.; Vecchio, L.; Barabino, S.; Battistelli, S.; Martin, T.; Malatesta, M.; Biggiogera, M. 2008. Can a genetically modified organism-containing diet influence embry development? A preliminary study on preimplantation mouse embryos. *European Journal of Histochemistry*, vol. 52 issue 4 (October-December):263-267.

In eukaryotic cells, pre-mRNAs undergo several transformation steps to generate mature mRNAs. Recent studies have demonstrated that a diet containing a genetically modified (GM) soybean can induce modifications of nuclear constituents involved in RNA processing in some tissues of young, adult and old mice. On this basis, we have investigated the ultrastructural and immunocytochemical features of pre-implantation embryos from mice fed either GM or non- GM soybean in order to verify whether the parental diet can affect the morpho-functional development of the embryonic ribonucleoprotein structural constituents involved in pre-mRNA pathways. Morphological observations revealed that the general aspect of embryo nuclear components is similar in the two experimental groups. However, immunocytochemical and in situ hybridization results suggest a temporary decrease of pre-mRNA transcription and splicing in 2-cell embryos and a resumption in 4-8-cell embryos from mice fed GM soybean; moreover, pre-mRNA maturation seems to be less efficient in both 2-cell and 4-8-cell embryos from GM-fed mice than in controls. Although our results are still preliminary and limited to the pre-implantation phases, the results of this study encourage deepening on the effects of food components and/or contaminants on embryo development.

http://www.ncbi.nlm.nih.gov/pubmed/19109102

Malatesta, M.; Boraldi, F.; Annovi, G.; Baldelli, B.; Battistelli, S.; Biggiogera, M.; Quaglino, D. 2008. A long-term study on female mice fed on a genetically modified soybean: effects on liver ageing. *Histochem Cell Biol*, 130, 967-77.

Liver represents a suitable model for monitoring the effects of a diet, due to its key role in controlling the whole metabolism. Although no direct evidence has been reported so far that genetically modified (GM) food may affect health, previous studies on hepatocytes from young female mice fed on GM soybean demonstrated nuclear modifications involving transcription and splicing pathways. In this study, the effects of this diet were studied on liver of old female mice in order to elucidate possible interference with ageing. The morpho-functional characteristics of the liver of 24-month-old mice, fed from weaning on control or GM soybean, were investigated by combining a proteomic approach with ultrastructural, morphometrical and immunoelectron microscopical analyses. Several proteins belonging to hepatocyte metabolism, stress response, calcium signalling and mitochondria were differentially expressed in GM-fed mice, indicating a more marked expression of senescence markers in comparison to controls. Moreover, hepatocytes of GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate. This study demonstrates that GM soybean intake can influence some liver features during ageing and, although the mechanisms remain unknown, underlines the importance to investigate the long-term consequences of GM-diets and the potential synergistic effects with ageing, xenobiotics and/or stress conditions.

http://www.ncbi.nlm.nih.gov/pubmed/18648843

Cirnatu, D.; Jompan, A.; Sin, A.; Zugravu, C. 2011. Multiple organ histopathological changes in broiler chickens fed on genetically modified organism. *Rom J Morphol Embryol*, 52 (1 Suppl): 475-480.

Diet can influence the structural characteristics of internal organs. An experiment involving 130 meat broilers was conducted during 42 days (life term for a meat broiler) to study the effect of feed with protein from genetically modified soy. The 1-day-old birds were randomly allocated to five study groups, fed with soy, sunflower, wheat, fish flour, PC starter. In the diet of each group, an amount of protein from soy was replaced with genetically modified soy (I - 0%, II - 25%, III - 50%, IV - 75%, V - 100% protein from genetically modified soy). The level of protein in soy, either modified, or non-modified, was the same. Organs and carcass weights were measured at about 42 days of age of the birds and histopathology exams were performed during May-June 2009. No statistically significant differences were observed in mortality, growth performance variables or carcass and organ yields between broilers consuming diets produced with genetically modified soybean fractions and those consuming diets produced with near-isoline control soybean fractions. Inflammatory and degenerative liver lesions, muscle hypertrophy, hemorrhagic necrosis of bursa, kidney focal tubular necrosis, necrosis and superficial ulceration of bowel and pancreatic dystrophies were found in tissues from broilers fed on protein from genetically modified soy. Different types of lesions found in our study might be due to other causes (parasites, viral) superimposed but their presence exclusively in groups fed with modified soy raises some serious questions about the consequences of use of this type of feed.

http://www.ncbi.nlm.nih.gov/pubmed/21424096

Carman, J.; Vlieger, H.; Ver Steeg, L.; Sneller, V.; Robinson, G.; Clinch-Jones, C.; Haynes, J.; Edwards, J. 2013. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. *Journal of Organic Systems*, 8 (1): 38-54[59].

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[59] Esse estudo uso como base da dieta Geneticamente Modificada uma mistura de milho tolerante ao glifosato, milho Bt e soja tolerante ao glifosato. Por isto, essa referência consta em três itens dessa parte, sendo 1.4, 2.2.1 e 2.2.2.

> A significant number of genetically modified (GM) crops have been approved to enter human food and animal feed since 1996, including crops containing several GM genes 'stacked' into the one plant. We randomised and fed isowean pigs (N=168) either a mixed GM soy and GM corn (maize) diet (N=84) or an equivalent non-GM diet (N=84) in a longterm toxicology study of 22.7 weeks (the normal lifespan of a commercial pig from weaning to slaughter). Equal numbers of male and female pigs were present in each group. The GM corn contained double and triple-stacked varieties. Feed intake, weight gain, mortality and blood biochemistry were measured. Organ weights and pathology were determined post-mortem. There were no differences between pigs fed the GM and non-GM diets for feed intake, weight gain, mortality, and routine blood biochemistry measurements. The GM diet was associated with gastric and uterine differences in pigs. GM-fed pigs had uteri that were 25% heavier than non-GM fed pigs (p=0.025). GM-fed pigs had a higher rate of severe stomach inflammation with a rate of 32% of GM-fed pigs compared to 12% of non-GM-fed pigs (p=0.004). The severe stomach inflammation was worse in GM-fed males compared to non-GM fed males by a factor of 4.0 (p=0.041), and GM-fed females compared to non-GM fed females by a factor of 2.2 (p=0.034).
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> Artigo completo disponível em http://www.organic-systems.org/journal/81/8106.pdf

2.2.2 Milho tolerante a herbicidas à base de glifosato

Após a liberação da soja RR, foram autorizados o plantio e o consumo de variedades de milho GM, tolerantes a herbicidas à base de glifosato. As implicações se mostraram mais relevantes na medida em que, no consumo do milho, existem casos de ingesta com processamento extremamente simples, envolvendo colheita de grãos antes da maturação e, portanto, com mais possibilidades de conter resíduos de agrotóxicos. Em vista disso, acumularam-se estudos importantes cobrindo espaços não avaliados para eventos com a soja GM.

Entre estes, talvez o mais polêmico foi publicado no final de 2012. Trata-se de avaliação do milho RR – NK603, envolvendo estudo de longo prazo e adotando protocolos similares àqueles utilizados pela Monsanto para argumentar ausência de riscos ao consumo daquela PGM. No centro da controvérsia (para mais informações sobre essa polêmica ver artigos da Parte 5 item 4.2.3) está o fato de o professor Séralini e sua equipe apontarem danos crônicos toxicológicos significativos e desenvolvimento de tumores com taxa alarmante, em consequência de ingesta continuada do NK603 por períodos superiores aos adotados em estudos anteriores.

Hammond[60], B.; Dudekb, R.; Lemen, J.; Nemeth, M. 2004. Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. *Food and Chemical Toxicology*, 42, 1003–1014.

The current study presents the results of a 13 week feeding study in rats with grain from Roundup Ready corn which is tolerant to the herbicide glyphosate. Herbicide tolerance was accomplished through the introduction of cp4 epsps coding sequences into the corn genome for in planta production of CP4 EPSPS enzymes. Unlike related corn EPSPS enzymes, CP4 EPSPS enzymes are not inhibited by the herbicide glyphosate. Purina TestDiets formulated Roundup Ready corn grain into rodent diets at levels of 11 and 33% (w/w). The responses of rats fed diets containing Roundup Ready corn grain were compared to that of rats fed diets containing non-transgenic grain (controls). All diets were nutritionally balanced and conformed to Purina Mills, Inc. specifications for Certified LabDiet 5002. There were 400 rats in the study divided into 10 groups of 20 rats/sex/group. Overall health, body weight, food consumption, clinical pathology parameters (hematology, blood chemistry, urinalysis), organ weights, gross and microscopic appearance of tissues were comparable between groups fed diets containing Roundup Ready and control corn grain. This study complements extensive agronomic, compositional and farm animal feeding studies with Roundup Ready corn grain, confirming it is as safe and nutritious as existing commercial corn hybrids.

http://www.ncbi.nlm.nih.gov/pubmed/15110110

REV - Vendômois, J.; Roullier, F.; Cellier, D.; Serálini, G-E. 2009. A comparison of the effects of three GM corn varieties on mammalian health. *International Journal of Biological Sciences*, 5, 706–726.

We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been modified to be tolerant to the broad spectrum herbicide Roundup and thus contains residues of this formulation. MON 810 and MON 863 are engineered to synthesize two different Bt toxins used as insecticides. Approximately 60 different biochemical parameters were classified per organ and measured in serum and urine after 5 and 14 weeks of feeding. GM maize-fed rats were compared first to their respective isogenic or parental non-GM equivalent control groups. This was followed by comparison to six reference groups, which had consumed various other non-GM maize varieties. We applied nonparametric methods, including multiple pairwise comparisons with a False Discovery Rate approach. Principal Component Analysis allowed the investigation of scattering of different factors (sex, weeks of feeding, diet, dose and group). Our analysis clearly reveals for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also noticed in the heart, adrenal glands, spleen and haematopoietic system. We conclude that these data highlight signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM corn. In addition, unintended direct or indirect metabolic consequences of the genetic modification cannot be excluded.

Artigo completo disponível em http://www.ijbs.com/v05p0706.htm

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[60] O que não aparece no resumo desse estudo, mas que justifica plenamente a sua inclusão nessa publicação, diz respeito as diferenças estatisticamente significativas observadas durante o experimento. Conforme ressaltado pelo Centro de Pesquisa e de Informação Independente em Engenharia Genética (CRIIGEN, 2007), cerca de 70 (67) diferenças estatísticas significativas foram observadas e relatadas pela empresa que comercializa a PGM, sendo: 12 relativas aos parâmetros hematológicos (hematócrito, plaquetas, neutrófilos, linfócitos, monócitos, volume corpuscular médio, concentração corpuscular média de hemoglobina); 18 relativas aos parâmetros químicos clínicos (albumina, nitrogênio uréico do sangue, creatinina, fósforo, sódio, cloreto, fosfatase alcalina, cálcio, potássio); 9 relativas a parâmetros químicos urinários (creatinina, fósforo, potássio, turgescencia da creatinina, pH, cálcio); 6 relativas ao peso dos órgãos (coração, cérebro, fígado); 14 nos pesos corporais e modificações de peso, e 8 relativas aos índices de consumo alimentar dos animais avaliados.

Séralini, G-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Vendomois, J. 2012. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food Chem Toxicol*, 50, 4221– 4231.

Retracted.

http://www.ncbi.nlm.nih.gov/pubmed/24490213

COM – Séralini, G-E.; Mesnage, R.; Defarge, N.; Gress, S.; Hennequin, D.; Clair, E.; Malatesta, M.; Vendômois, J 2013. Answers to critics: Why there is a long term toxicity due to Roundup-tolerant genetically modified maize and to a Roundup herbicide. *Food and Chemical Toxicology*, 53: 476-83.

Our recent work (Séralini et al., 2012) remains to date the most detailed study involving the life-long consumption of an agricultural genetically modified organism (GMO). This is true especially for NK603 maize for which only a 90-day test for commercial release was previously conducted using the same rat strain (Hammond et al., 2004). It is also the first long term detailed research on mammals exposed to a highly diluted pesticide in its total formulation with adjuvants. This may explain why 75% of our first criticisms arising within a week, among publishing authors, come from plant biologists, some developing patents on GMOs, even if it was a toxicological paper on mammals, and from Monsanto Company who owns both the NK603 GM maize and Roundup herbicide (R). Our study has limits like any one, and here we carefully answer to all criticisms from agencies, consultants and scientists, that were sent to the Editor or to ourselves. At this level, a full debate is biased if the toxicity tests on mammals of NK603 and R obtained by Monsanto Company remain confidential and thus unavailable in an electronic format for the whole scientific community to conduct independent scrutiny of the raw data. In our article, the conclusions of long-term NK603 and Roundup toxicities came from the statistically highly discriminant findings at the biochemical level in treated groups in comparison to controls, because these findings do correspond in an blinded analysis to the pathologies observed in organs, that were in turn linked to the deaths by anatomopathologists. GM NK603 and R cannot be regarded as safe to date.

Artigo completo disponível em http://www.gmoseralini.org/wp-content/uploads/2013/01/Seralinial-AnswersCritics-FCT_2013.pdf

Carman, J.; Vlieger, H.; Ver Steeg, L.; Sneller, V.; Robinson, G.; Clinch-Jones, C.; Haynes, J.; Edwards, J. 2013. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. *Journal of Organic Systems*, 8 (1): 38-54[61].

A significant number of genetically modified (GM) crops have been approved to enter human food and animal feed since 1996, including crops containing several GM genes 'stacked' into the one plant. We randomised and fed isowean pigs (N=168) either a mixed GM soy and GM corn (maize) diet (N=84) or an equivalent non-GM diet (N=84) in a longterm toxicology study of 22.7 weeks (the normal lifespan of a commercial pig from weaning to slaughter). Equal numbers of male and female pigs were present in each group. The GM corn contained double and triple-stacked varieties. Feed intake, weight gain, mortality and blood biochemistry were measured. Organ weights and pathology were determined post-mortem. There were no differences between pigs fed the GM and non-GM diets for feed intake, weight gain, mortality, and routine blood biochemistry measurements. The GM diet was associated with gastric and uterine differences in pigs. GM-fed

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[61] Esse estudo uso como base da dieta Geneticamente Modificada uma mistura de milho tolerante ao glifosato, milho Bt e soja tolerante ao glifosato. Por isto, essa referência consta em três itens dessa parte, sendo 1.4, 2.2.1 e 2.2.2.

pigs had uteri that were 25% heavier than non-GM fed pigs (p=0.025). GM-fed pigs had a higher rate of severe stomach inflammation with a rate of 32% of GM-fed pigs compared to 12% of non-GM-fed pigs (p=0.004). The severe stomach inflammation was worse in GM-fed males compared to non-GM fed males by a factor of 4.0 (p=0.041), and GM-fed females compared to non-GM fed females by a factor of 2.2 (p=0.034).

Artigo completo disponível em http://www.organic-systems.org/journal/81/8106.pdf

Séralini, G-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Vendômois, J. 2014. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26:14.

Background: The health effects of a Roundup-tolerant NK603 genetically modified (GM) maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 ppb of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in rats. This study constitutes a follow-up investigation of a 90-day feeding study conducted by Monsanto in order to obtain commercial release of this GMO, employing the same rat strain and analyzing biochemical parameters on the same number of animals per group as our investigation. Our research represents the first chronic study on these substances, in which all observations including tumors are reported chronologically. Thus, it was not designed as a carcinogenicity study. We report the major findings with 34 organs observed and 56 parameters analyzed at 11 time points for most organs.

Results: Biochemical analyses confirmed very significant chronic kidney deficiencies, for all treatments and both sexes; 76% of the altered parameters were kidney-related. In treated males, liver congestions and necrosis were 2.5 to 5.5 times higher. Marked and severe nephropathies were also generally 1.3 to 2.3 times greater. In females, all treatment groups showed a two- to threefold increase in mortality, and deaths were earlier. This difference was also evident in three male groups fed with GM maize. All results were hormone- and sex-dependent, and the pathological profiles were comparable. Females developed large mammary tumors more frequently and before controls; the pituitary was the second most disabled organ; the sex hormonal balance was modified by consumption of GM maize and Roundup treatments. Males presented up to four times more large palpable tumors starting 600 days earlier than in the control group, in which only one tumor was noted. These results may be explained by not only the non-linear endocrine-disrupting effects of Roundup but also by the overexpression of the EPSPS transgene or other mutational effects in the GM maize and their metabolic consequences.

Conclusion: Our findings imply that long-term (2 year) feeding trials need to be conducted to thoroughly evaluate the safety of GM foods and pesticides in their full commercial formulations.

Artigo completo disponível em http://www.enveurope.com/content/26/1/14

3 Outras plantas transgênicas[62] em fase de avaliação ou com comercialização suspensa

Publicações científicas aplicadas à avaliação de riscos com plantas transgênicas cobrem além das espécies dominantes (como milho e soja contendo tecnologias Bt e TH), eventos novos ou de escassa abrangência, já disponibilizados em alguns mercados, com processo de comercialização cancelado ou ainda em fase de avaliação.

Os artigos discutem alguns desses casos novos – a exemplo do arroz e da batata – e também eventos com milho e soja, que sofreram outros tipos de modificações genéticas –, apontando riscos para a saúde humana e/ou animal.

A literatura científica também oferece artigos[63] onde os autores consideram não relevantes sob o ponto de vista biológico diferenças estatisticamente significativas encontradas nas comparações entre grupos de animais alimentados com PGMs e grupos de animais controle. Os autores dessa sistematização entendem como importantes – e abertas para interpretações diversas – essas observações

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[62] Neste ponto cabe lembrar o histórico de uso não seguro de produtos transgênicos sintetizados em micro-organismos GM. No final dos anos 1980, associou-se o aparecimento da doença chamada EMS (*Eosinophilia-Myalgia-Syndrome*) ao uso do L-triptofano produzido por uma bactéria transgênica comercializada pela empresa japonesa Showa Denko K.K. Houve contabilização de aproximadamente 40 casos mortais e de 1500 pessoas com graves distúrbios neurológicos, em sua maioria irreversíveis. Apesar de a indústria apontar a responsabilidade de danos para constituintes químicos indesejados, envolvidos no processo de filtração do L-Triptofano, (notadamente IMT e EBT), não pode ser descartada a hipótese de que o uso de bactérias transgênicas estivesse envolvido. Isso porque alguns casos de intoxicação surgiram antes de o processo de filtração ser aplicado na empresa japonesa em foco. Ademais, os constituintes se revelaram similares – mas não idênticos – ao L-Triptofano. A possibilidade de organismos transgênicos produzirem substâncias não desejadas está explicitada no item 2 da Parte 1. Mais informações sobre o caso do L-Triptofano podem ser encontradas em Belongia et al., 1990 (*An investigation of the cause of the eosinophilia-myalgia syndrome associated with tryptophan use*. N Engl J Med. Aug 9 1990; 323(6): 357-365); Kilbourne et al., 1996 (Tryptophan produced by Showa Denko and epidemic eosinophilia-myalgia syndrome. *J Rheumatol Suppl*. 1996 Oct;46:81-8; discussion 89-91) e Müller et al., 1999 (Synthesis and formation of an EMS correlated contaminant in biotechnologically manufactured L-tryptophan. *Adv Exp Med Biol*. 1999;467:481-6).

[63] Tais como Appenzeller et al, 2008 (Subchronic feeding study of herbicide-tolerant soybean DP-356O43-5 in Sprague-Dawley rats. *Food Chem Toxicol.* 2008, 46: 2201-2213) e Appenzeller et al., 2009 (Subchronic feeding study of grain from herbicide-tolerant maize DPO9814O- 6 in prague-Dawley rats. *Food Chem Toxicol.* 2009, 47: 2269-2280).

estatísticas, e tal contradição é argumento relevante para realização de novos e mais detalhados estudos[64].

Nordlee, J.; Taylor, S.; Townsend, J.; Thomas, L.; Bush, R. 1996. Identification of a Brazil-Nut allergen in transgenic soybeans. *The New England Journal of Medicine*, 334: 668-92.

Background: The nutritional quality of soybeans (Glycine max) is compromised by a relative deficiency of methionine in the protein fraction of the seeds. To improve the nutritional quality, methionine-rich 2S albumin from the Brazil nut (Betholletia excelsa) has been introduced into transgenic soybeans. Since the Brazil nut is a known allergenic food, we assessed the allergenicity of the 2S albumin.
Methods: The ability of proteins in transgenic and non-transgenic soybeans, Brazil nuts, and purified 2S albumin to bind to IgE in serum from subjects allergic to Brazil nuts was determined by radioallergosorbent tests (4 subjects) and sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (9 subjects) with immunoblotting and autoradiography. Three subjects also underwent skin-prick testing with extracts of soybean, transgenic soybean, and Brazil nut.
Results: On radioallergosorbent testing of pooled serum from four subjects allergic to Brazil nuts, protein extracts of transgenic soybean inhibited binding of IgE to Brazil-nut proteins. On immunoblotting, serum IgE from eight of nine subjects bound to purified 2S albumin from the Brazil nut and the transgenic soybean. On skin-prick testing, three subjects had positive reactions to extracts of Brazil nut and transgenic soybean and negative reactions to soybean extract.
Conclusions: The 2S albumin is probably a major Brazil-nut allergen, and the transgenic soybeans analyzed in this study contain this protein. Our study show that an allergen from a food known to be allergenic can be transferred into another food by genetic engineering.

Artigo completo disponível em http://www.nejm.org/doi/full/10.1056/NEJM199603143341103#Top

Ewen, S.; Pusztai, A. 1999. Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. *Lancet*, 354: 1353-4.

Diets containing genetically modified (GM) potatoes expressing the lectin Galanthus nivalis agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

http://www.ncbi.nlm.nih.gov/pubmed/10533866

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[64] Entre essas plantas menos estudadas, vale destacar o caso dos eventos estaqueados, que agregam – por melhoramento convencional – dois ou mais transgenes. Cabe salientar a escassez de estudos publicados – na literatura científica – sobre essas PGMs, com o gravame de que a maioria destes são realizados pelas empresas de biotecnologia ou a seu serviço. Ver MacKenzie et al., 2007 (Thirteen week feeding study with transgenic maize grain containing event DAS-O15O7-1 in Sprague-Dawley rats. *Food Chem Toxicol* 2007, 45:551-562); Malley et al., 2007 (Subchronic feeding study of DAS-59122-7 maize grain in Sprague-Dawley rats. *Food Ch*em Toxicol 2007, 45:1277-1292); He et al., 2008 (Comparison of grain from corn rootworm resistant transgenic DAS-59122-7 maize with nontransgenic maize grain in a 90-day feeding study in Sprague-Dawley rats. Food Chem Toxicol 2008, 46:1994-2002); Appenzeller et al., 2009 (Subchronic feeding study with genetically modified stacked trait lepidopteran and coleopteran resistant (DAS-O15O7-1xDAS-59122-7) maize grain in Sprague-Dawley rats. Food Chem Toxicol 2009, 47:1512-1520). Todos estes constatam diferenças estatisticamente significativas e as desprezam ao concluir por ausência de risco biológico.

Schrøder, M.; Wilcks, A.; Jacobsen, H.; Miller, A.; Frenzel, T.; Danier, J.; Rychlik, M.; Shu, Q.; Emami, K.; Sudhakar, D.; Gatehouse, A.; Engel, K-H.; Knudsen, I. 2007. A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin *Galanthus nivalis* (GNA). *Food and Chemical Toxicology*, Volume 45, Issue 3, Pages 350-363.

Genetically modified plants expressing insecticidal traits offer a new strategy for crop protection, but at the same time present a challenge in terms of food safety assessment. The present 90-day feeding study was designed to assess the safety of a rice variety expressing the snowdrop Galanthus nivalis lectin (GNA lectin), and forms part of a EU-funded project where the objective has been to develop and validate sensitive and specific methods to assess the safety of genetically modified foods. Male and female Wistar rats were given a purified diet containing either 60% genetically modified or parental rice for 90 days. This corresponds to a mean daily GNA lectin intake of approximately 58 and 67mg/kg body weight for males and females, respectively. Prior to the animal study comprehensive analytical characterization of both rice materials was performed. The chemical analyses showed a number of statistically significant differences, with the majority being within the ranges reported in the literature. In the animal study a range of clinical, biological, immunological, microbiological and pathological parameters were examined. A number of significant differences were seen between groups fed the two diets, but none of them were considered to be adverse. In conclusion, the design of the present animal study did not enable us to conclude on the safety of the GM food. Additional group(s) where the expressed gene products have been spiked to the diet should be included in order to be able to distinguish whether the observed effects were due to the GNA lectin per se or to secondary changes in the GM rice.

Artigo completo disponível em http://stopogm.net/sites/stopogm.net/files/webfm/plataforma/Poulsen2006_rice.pdf

Krzyzowska, M.; Wincenciak, M.; Winnicka, A.; Baranowski, A.; Jaszczak, K.; Zimny, J.; Niemiałtowski, M. 2010. The effect of multigenerational diet containing genetically modified triticale on immune system in mice. *Polish Journal of Veterinarium Sciences*, 13:423-30.

The safety assessment of genetically modified (GM) food and feed is performed to identify the possible effects upon animal and human health, also the long-term, multigenerational influence upon functioning of different organs and systems, such as the immune system. In this study C57BL/6J mice were fed for five consecutive generations with pellets containing 20% of conventional triticale grain (control) vs. pellets containing 20% of the transgenic triticale grain resistant to BASTA herbicide (experimental). The F5 experimental animals showed enlarged inguinal and axillary lymph nodes, but not spleens, and increased WBC counts in blood (but within the norm for Mus musculus). Immunophenotyped cell suspensions derived from spleens, inguinal and axillaris lymph nodes and PBMCs from blood showed the significant decrease in the percentage of T cells in spleen and lymph nodes and the B cells in lymph nodes and blood of the F5 experimental mice in comparison to the control F5 mice. Immunoblotting analysis of IL-2, IL-4, IL-10, IL-12, IL- 6, IFN-gamma levels in serum showed significantly increased IL-2 levels and decreased IL-6 levels in the F5-experimental mice sera. No significant changes in the levels of IgE in sera in both mice groups were observed. The obtained results indicate that multigenerational use of feeds for rodents containing the GM-triticale leads to expansion of the B cell compartment in the secondary lymphoid organs, but it is not caused by malignant processes or the allergic response.

http://www.ncbi.nlm.nih.gov/pubmed/21033555

4 Riscos associados ao consumo de transgenes e de pequenos RNA não codificadores (sRNAs/ncRNA)

Para além do efeito direto da(s) proteína(s) recombinante(s) produzida(s) por plantas transgênicas, suscetíveis de possuir atividade alergênica e/ou tóxica, há que examinar efeitos de interações entre o(s) transgene(s) (ou outros produtos biológicos não codificadores de proteína, como diversos tipos de RNA[65]) e os organismos que o consomem.

Evidentemente as preocupações tendem a crescer quando as modificações envolvem transgenes com função biológica chave, capazes de intervir sobre outros aspectos de expressão gênica, no organismo que o consome.

4.1 Sobrevivência de (trans)gene à digestão e sua circulação no sangue

De maneira geral, a indústria descarta riscos associados às interações do transgene com o organismo consumidor. Para tanto, argumenta que o DNA se faz completamente degradado ao longo do processo digestivo.

Contrariando essa premissa os estudos a seguir observam que sequências de DNA (gene ou transgene) não apenas sobrevivem à digestão como circulam no sangue.

A reversibilidade da presença de DNA recombinante no organismo consumidor, após períodos de dieta sem o OGM – tal como salientado por alguns dos autores abaixo referidos – não permite concluir pela ausência de riscos em termos de longo prazo.

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[65] O feijão transgênico evento 5.1 desenvolvido pela Embrapa e liberado comercialmente em 2011 no Brasil, objetivava impedir a reprodução, na planta, do vírus do mosaico dourado do feijoeiro. Para tanto, recorria ao mecanismo do RNA interferente, levando ao silenciamento gênico de um comando genético relacionado à replicação viral (envolvendo moléculas de ncRNA, notadamente de dsRNA e siRNA).

Schubbert, R.; Rentz, D.; Schmitzx, B.; Doerfler, W. 1997. Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *PNAS*, 94, 961-6 (1).

Food-ingested foreign DNA is not completely degraded in the gastrointestinal tract of mice. Phage M13mp18 DNA as a test molecule devoid of homology to mouse DNA was pipette-fed to or added to the food supply of mice. The fate of this foreign DNA in the animals was followed by several methods. In 84 animals, fragments of M13mp18 DNA were detected in the contents of the small intestine, the cecum (until 18 h), the large intestine, or the feces. In 254 animals, M13mp18 DNA fragments of up to 976 bp were found in blood 2-8 h after feeding. In buffer-fed control animals, M13mp18 DNA could not be detected. M13mp18 DNA fragments were traced by PCR in peripheral leukocytes and located by fluorescent in situ hybridization in about 1 of 1000 white cells between 2 and 8 h, and in spleen or liver cells up to 24 h after feeding, but not later. M13mp18 DNA could be traced by fluorescent in situ hybridization in the columnar epithelial cells, in the leukocytes in Peyer's patches of the cecum wall, in liver cells, and in B cells, T cells, and macrophages from spleen. These findings suggest transport of foreign DNA through the intestinal wall and Peyer's patches to peripheral blood leukocytes and into several organs. Upon extended feeding, M13mp18 DNA could be recloned from total spleen DNA into a lambda vector. Among about 2.5 x 10(7) lambda plaques, one plaque was isolated that contained a 1299 nucleotide pair fragment (nt 4736-6034) of sequence-identified M13mp18 DNA. This fragment was covalently linked to an 80 nt DNA segment with 70% homology to the mouse IgE receptor gene. The DNA from another lambda plaque also contained mouse DNA, bacterial DNA, and rearranged lambda DNA. Two additional plaques contained M13mp18 DNA fragments of at least 641 (nt 2660-3300) or 794 (nt 4640-5433) nucleotide pairs. The medical and evolutionary implications of these observations may be considerable.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC19622/

Schubbert, R.; Hohlweg, U.; Renz, D.; Doerfler, W. 1998. On the fate of orally ingested foreign DNA in mice: chromosal association and placental transmission to the fetus. *Molecular Genomics and Genetics,* 259 (6): 569-76.

We have previously shown that, when administered orally to mice, bacteriophage M13 DNA, as a paradigm foreign DNA without homology to the mouse genome, can persist in fragmented form in the gastrointestinal tract, penetrate the intestinal wall, and reach the nuclei of leukocytes, spleen and liver cells. Similar results were obtained when a plasmid containing the gene for the green fluorescent protein (pEGFP-C1) was fed to mice. In spleen, the foreign DNA was detected in covalent linkage to DNA with a high degree of homology to mouse genes, perhaps pseudogenes, or to authentic E. coli DNA. We have now extended these studies to the offspring of mice that were fed regularly during pregnancy with a daily dose of 50 microg of M13 or pEGFP-C1 DNA. Using the polymerase chain reaction (PCR) or the fluorescent in situ hybridization (FISH) method, foreign DNA, orally ingested by pregnant mice, can be discovered in various organs of fetuses and of newborn animals. The M13 DNA fragments have a length of about 830 bp. In various organs of the mouse fetus, clusters of cells contain foreign DNA as revealed by FISH. The foreign DNA is invariably located in the nuclei. We have never found all cells of the fetus to be transgenic for the foreign DNA. This distribution pattern argues for a transplacental pathway rather than for germline transmission which might be expected only after long-time feeding regimens. In rare cells of three different fetuses, whose mothers have been fed with M 13 DNA during gestation, the foreign DNA was detected by FISH in association with both chromatids. Is maternally ingested foreign DNA a potential mutagen for the developing fetus?

http://www.ncbi.nlm.nih.gov/pubmed/9819049

Martín-Orúe, S.; O'Donnell, A.; Ariño, J.; Netherwood, T.; Gilbert H.; Mathers, J. 2002. Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. *British Journal of Nutrition*, 87, 533-542.

The inclusion of genetically modified (GM) foods in the human diet has caused considerable debate. There is concern that the transfer of plant-derived transgenes to the resident intestinal microflora could have safety implications. For these gene transfer events to occur, the nucleic acid would need to survive passage through the gastrointestinal tract. The aim of the present study was to evaluate the rate at which transgenes, contained within GM soya and maize, are degraded in gastric and small bowel simulations. The data showed that 80 % of the transgene in naked GM soya DNA was degraded in the gastric simulations, while no degradation of the transgenes contained within GM soya and maize were observed in these acidic conditions. In the small intestinal simulations, transgenes in naked soya DNA were degraded at a similar rate to the material in the soya protein. After incubation for 30 min, the transgenes remaining in soya protein and naked DNA were 52 (sem 13.1) % and 34 (sem 17.5) %, respectively, and at the completion of the experiment (3 h) these values were 5 % and 3 %, respectively. In contrast to the soya transgene, the maize nucleic acid was hydrolysed in the small intestinal simulations in a biphasic process in which approximately 85 % was rapidly degraded, while the rest of the DNA was cleaved at a rate similar to that in the soya material. Guar gum and tannic acid, molecules that are known to inhibit digestive enzymes, did not influence the rate of transgene degradation in soya protein. In contrast guar gum reduced the rate of transgene degradation in naked soya DNA in the initial stages, but the polysaccharide did not influence the amount of nucleic acid remaining at the end of the experiment. Tannic acid reduced the rate of DNA degradation throughout the small bowel simulations, with 21 (sem 5.4) % and 2 (sem 1.8) % of the naked soya DNA remaining in the presence and absence of the phenolic acid, respectively. These data indicate that some transgenes in GM foods may survive passage through the small intestine.

http://www.ncbi.nlm.nih.gov/pubmed/12067423

Netherwood, T.; Martín-Orúe, S.; O'Donnell, A.; Gockling, S.; Graham, J.; Mathers, J.; Gilbert, H. 2004. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature Biotechnology*, 22, 204-209.

The inclusion of genetically modified (GM) plants in the human diet has raised concerns about the possible transfer of transgenes from GM plants to intestinal microflora and enterocytes. The persistence in the human gut of DNA from dietary GM plants is unknown. Here we study the survival of the transgene *epsps* from GM soya in the small intestine of human ileostomists (i.e., individuals in which the terminal ileum is resected and digesta are diverted from the body via a stoma to a colostomy bag). The amount of transgene that survived passage through the small bowel varied among individuals, with a maximum of 3.7% recovered at the stoma of one individual. The transgene did not survive passage through the intact gastrointestinal tract of human subjects fed GM soya. Three of seven ileostomists showed evidence of low-frequency gene transfer from GM soya to the microflora of the small bowel before their involvement in these experiments. As this low level of *epsps* in the intestinal microflora did not increase after consumption of the meal containing GM soya, we conclude that gene transfer did not occur during the feeding experiment.

http://www.nature.com/nbt/journal/v22/n2/abs/nbt934.html

Mazza, R.; Soave, M.; Morlacchini, M.; Piva, G.; Marocco, A. 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. *Transgenic Research*, 14:775–784.

In Europe, public and scientific concerns about the environmental and food safety of GM (Genetically Modified) crops overshadow the potential benefits offered by crop biotechnology to improve food quality. One of the concerns regarding the use of GM food in human and animal nutrition is the effect that newly introduced sequences may have on the organism. In this paper, we assess the potential transfer of diet-derived DNA to animal tissues after consumption of GM plants. Blood, spleen, liver, kidney and muscle tissues from piglets fed for 35 days with diets containing either GM (MON810) or a conventional maize were investigated for the presence of plant DNA. Only fragments of specific maize genes (Zein, Sh-2) could be detected with different frequencies in all the examined tissues except muscle. A small fragment of the Cry1A(b) transgene was detected in blood, liver, spleen and kidney of the animals raised with the transgenic feed. The intact Cry1A(b) gene or its minimal functional unit were never detected. Statistical analysis of the results showed no difference in recovery of positives for the presence of plant DNA between animals raised with the transgenic feed and animals raised with the conventional feed, indicating that DNA transfer may occur independently from the source and the type of the gene. From the data obtained, we consider it unlikely that the occurrence of genetic transfer associated with GM plants is higher than that from conventional plants.

http://www.ncbi.nlm.nih.gov/pubmed/16245168

Tudisco, R.; Infascelli, F.; Cutrignelli, M.; Bovera, F.; Morcia, C.; Facciolli, P.; Terzi, V. 2006. Fate of feed plant DNA monitored in water buffalo (*Bubalus bubalis*) and rabbit (*Oryctolagus cuniculus*). *Livestock Science*, Vol. 105 (1-3): 12-18.

The effect of the digestion process in the gastro-intestinal tract (GIT) of animal models on the fate and integrity of plant DNA has been widely evaluated since DNA availability and integrity is a key factor for hypothetical horizontal gene transfer of recombinant DNA from GM crop-derived feeds to animal and human gut microflora. In this study, plant DNA sequences from high and low copy number genes were monitored in GIT and tissues of buffaloes and rabbits. Using a real-time PCR approach to track plant DNA in animal samples, we demonstrated the persistence of fragmented plant DNA blood and tissues of buffaloes and rabbits raised with conventional feeding.

http://www.livestockscience.com/article/S1871-1413(06)00227-7/abstract

Sharma, R.; Damgaard, D.; Alexander, T.; Dugan, M.; Aalhus, J.; Stanford, K.; McAllister, T. 2006. Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready Canola meal. *J Agric Food Chem*, 54 (5): 1699-1709.

The persistence of plant-derived recombinant DNA in sheep and pigs fed genetically modified (Roundup Ready) canola was assessed by PCR and Southern hybridization analysis of DNA extracted from digesta, gastrointestinal (GI) tract tissues, and visceral organs. Sheep (n = 11) and pigs (n = 36) were fed to slaughter on diets containing 6.5 or 15% Roundup Ready canola. Native plant DNA (high- and low-copy-number gene fragments) and the cp4 epsps transgene that encodes 5-enolpyruvyl shikimate-3-phosphate synthase were tracked in ruminal, abomasal, and large intestinal digesta and in tissue from the esophagus, rumen, abomasum, small and large intestine, liver, and kidney of sheep and in cecal content and tissue from the duodenum, cecum, liver, spleen, and kidney of pigs. High-copy chloroplast-specific DNA (a 520-bp fragment) was detected in all digesta samples, the majority (89-100%) of intestinal tissues, and at least one of each visceral organ sample (frequencies of 3-27%) from sheep and swine. Low-copy rubisco fragments (186- and 540-bp sequences from the small subunit) were present at slightly lower, variable frequencies in digesta (18-82%) and intestinal tissues (9-27% of ovine and 17-25% of porcine samples) and infrequently in visceral organs (1 of 88 ovine samples; 3 of 216 porcine samples). Each of the five cp4 epsps transgene fragments (179-527 bp) surveyed was present in at least 27% of ovine large intestinal content samples (maximum = 64%) and at least 33% of porcine cecal content samples (maximum = 75%). In sheep, transgene fragments were more common in intestinal digesta than in ruminal or abomasal content. Transgene fragments were detected in 0 (esophagus) to 3 (large intestine) GI tract tissues from the 11 sheep and in 0-10 of the duodenal and cecal tissues collected from 36 pigs.

The feed-ingested recombinant DNA was not detected in visceral tissues (liver, kidney) of lambs or in the spleen from pigs. Of note, however, one liver and one kidney sample from the pigs (different animals) were positive for a 278-bp fragment of the transgenic cp4 epsps (denoted F3). Examination of genomic libraries from these tissues yielded no conclusive information regarding integration of the fragment into porcine DNA. This study confirms that feed-ingested DNA fragments (endogenous and transgenic) do survive to the terminal GI tract and that uptake into gut epithelial tissues does occur. A very low frequency of transmittance to visceral tissue was confirmed in pigs, but not in sheep. It is recognized that the low copy number of transgenes in GM feeds is a challenge to their detection in tissues, but there was no evidence to suggest that recombinant DNA would be processed in the gut in any manner different from endogenous feed-ingested genetic material.

http://www.ncbi.nlm.nih.gov/pubmed/16506822

Agodi, A.; Barchitta, M.; Grillo, A.; Sciacca, S. 2006. Detection of genetically modified DNA sequences in milk from the Italian market. *Int J Hyg Environ Health*, 209: 81-88.

The possible transfer and accumulation of novel DNA and/or proteins in food for human consumption derived from animals receiving genetically modified (GM) feed is at present the object of scientific dispute. A number of studies failed to identify GM DNA in milk, meat, or eggs derived from livestock receiving GM feed ingredients. The present study was performed in order to: (i) develop a valid protocol by PCR and multicomponent analysis for the detection of specific DNA sequences in milk, focused on GM maize and GM soybean; (ii) assess the stability of transgenic DNA after pasteurization treatment and (iii) determine the presence of GM DNA sequences in milk samples collected from the Italian market. Results from the screening of 60 samples of 12 different milk brands demonstrated the presence of GM maize sequences in 15 (25%) and of GM soybean sequences in 7 samples (11.7%). Our screening methodology shows a very high sensitivity and the use of an automatic identification of the amplified products increases its specificity and reliability. Moreover, we demonstrated that the pasteurization process is not able to degrade the DNA sequences in spiked milk samples. The detection of GM DNA in milk can be interpreted as an indicator of fecal or airborne contamination, respectively, with feed DNA or feed particles, although an alternative source of contamination, possibly recognizable in the natural environment can be suggested. Further studies, performed on a larger number of milk samples, are needed to understand the likely source of contamination of milk collected from the Italian market.

http://www.ncbi.nlm.nih.gov/pubmed/16373205

Chainark, P.; Satoh, S.; Hino, T.; Kiron, V.; Hirono, I.; Aoki, T. 2006. Availability of genetically modified soybean meal in rainbow trout *Oncorhynchus mykiss* diets. *Fisheries Science*, 72: 1072 – 1078.

The use of genetically modified defatted soybean meal (GM SBM) as rainbow trout feed was investigated, in comparison with non-GM SBM. Both SBMs were included at levels of approximately 15 and 30% in four diets (42% protein). The diets were fed to juvenile fish (48.3 g average weight) for 12 weeks. The nutrient use showed that there was no significant difference in growth and feed performance between GM and non-GM SBM groups at both inclusion levels at the end of 12th week. The cauliflower mosaic virus *35S*promoter fragment (220 bp) of the GM SBM was detected in the muscle of fish receiving both levels of GM SBM diet by nested PCR, but the frequency of detection was greater at the higher inclusion level. Additionally, the promoter fragment was not detected by the fifth day after changing the diet to non-GM. Conversely, the promoter fragment was not detected from fish fed with the non-GM SBM diet. The results demonstrated that the availability of protein in GM SBM was similar to that of non-GM SBM, and the promoter fragments found in the muscle of fish were not detectable after changing the diet to non-GM, verifying the availability of the GM SBM in rainbow trout feed.

http://onlinelibrary.wiley.com/doi/10.1111/j.1444-2906.2006.01258.x/abstract

Chainark, P.; Satoh, S.; Hirono, I.; Aoki, T.; Endo, M. 2008. Availability of genetically modified feed ingredient: investigations of ingested foreign DNA in rainbow trout *Oncorhynchus mykiss*. *Fisheries Science*, 74: 380-390.

Foreign DNA fragments from genetically modified defatted soybean meal (GM SEM) in rainbow trout was traced by nested polymerase chain reaction (PCR) and located by *in situ* hybridization. Either a GM or non-GM SBM formulated diet (42% protein) was fed to fish (average weight 50.5 g) for 2 weeks. The degradation results showed that the cauliflower mosaic virus *35S* promoter (220 bp) fragment was detected in the contents of digestive system only in fish fed the GM SBM diet, and it was not detected on the third day after changing the diet to the non-GM SBM diet. For the possible transferal results, the promoter fragment was detected in the leukocyte, head kidney and muscle only of fish fed the GM SBM diet; it was not detected on the fifth day after changing the diet to the non-GM SBM diet. These results suggest that a foreign DNA fragment was not completely degraded and might be taken up into organs through the gastrointestinal tract. However, foreign DNA was not detected after the withdrawal period. Thus, the data show that uptake of DNA from GM SBM might not remain in the tissues of fish fed GM SBM diet

http://link.springer.com/article/10.1111%2Fj.1444-2906.2008.01535.x

Ran, T.; Mei, L.; Lei, W.; Aihua, L.; Ru, H.; Jie, S. 2009. Detection of transgenic DNA in tilapias (*Oreochromis niloticus*, GIFT strain) fed genetically modified soybeans (Roundup Ready). *Aquaculture Research*, 40: 1350-1357.

We used nested-polymerase chain reaction (PCR) to detect Roundup Ready soybean in aquatic feeds and feeding tilapias. A template concentration of 10–10 g μL–1 DNA solution could be detected with a dilute degree of 0.01%. Most (90.6%) of the aquatic feeds containing soybean byproduct included exogenous DNA segments. We also compared genetically modified (GM) soybean with non-GM soybean diets in feeding tilapias (Oreochromis niloticus, GIFT strain) and examined the residual fragments (254 bp) of GM soybeans. Tilapias receiving GM soybean diets had DNA fragments in different tissues and organs, indicating that exogenous GM genes were absorbed systemically and not completely degraded by the tilapia's alimentary canal.

Artigo completo disponível em http://www.actualidadambiental.pe/wp-content/uploads/2009/09/Aquaculture-Research-2009-40-1350-1357.pdf

Tudisco, R.; Mastellone, V.; Cutrignelli, M.; Lombardi, P.; Bovera, F.; Mirabella, N.; Piccolo, G.; Calabro, S.; Avallone L.; Infascelli, F. 2010. Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. *The Animal Consortium*, 1-10; 4:1662-1671.

The presence of DNA fragments in blood and milk from goats fed conventional (control) or Roundup Ready® soybean meal solvent extracted (s.e.; treated) was investigated by using a polymerase chain reaction approach. The same investigation was carried out on blood, skeletal muscle and organs from kids of both groups fed only dams' milk until weaning. Moreover, the possible effects on cell metabolism were evaluated by determination of several specific enzymes in serum, heart, skeletal muscle, liver and kidney. Fragments of the multicopy chloroplast (trnL) gene were found in blood and milk samples from goats of both groups. In kids, the chloroplast fragments were found in samples of both groups. In samples, which proved positive for the presence of chloroplast DNA, fragments of the specific soybean single copy gene (lectin) were detected in several blood and milk samples. The same fragment was also found in control and treated groups of kids. Transgenic fragments were not found in those samples, which were found positive for chloroplast fragments of control groups of either goats or kids. On the contrary, in blood and milk of treated goats, fragments both of the 35S promoter and the CP4 epsps gene were detected. These fragments were also found in treated kids with a significant detection of the 35S promoter in liver, kidney and blood, and of the CP4 epsps gene fragment in liver, kidney, heart and muscle. A significant increase in lactic dehydrogenase, mainly concerning the lactic dehydrogenase-1 isoenzyme was found in heart, skeletal muscle and kidney of treated kids, thus suggesting a change in the local production of the enzyme. Finally, no significant differences were detected concerning kid body and organ weight.

Artigo completo disponível em http://journals.cambridge.org/download.php?file=%2FANM%2FANM4_10%2FS1751731110000728a.pdf&code=3095a03e5555a36bd69390f92ddf4fcd

Aris, A.; Leblanc, S. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, 31(4): 528-33. doi: 10.1016/j.reprotox.2011.02.004.

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

http://www.ncbi.nlm.nih.gov/pubmed/21338670

Grønsberg, I.; Nordgård, L.; Fenton, K.; Hegge, B.; Nielsen, K.; Bardocz, S.; Pusztai, A.; Traavik, T. 2011. Uptake and organ distribution of feed introduced plasmid DNA in growing or pregnant rats. *Food and Nutrition Sciences*, 2, 377-386.

Fragments of DNA present in food and feed are taken up by the gastrointestinal tract (GIT) of mammals. The extent of uptake varies according to organism, study design and DNA source. This study explores the hypothesis that actively growing, as well as pregnant rats, are more likely to take up DNA from the GIT than mature animals due to the high demand for nutrients for tissue and organ development. Plasmid DNA (pDNA) was added to standard feed for growing, and pregnant rats. The young rats received one pDNA (50 µg) containing meal by gavage. Blood, organ and tissue samples were harvested at 2 h to 3 days post feeding (p.f). The pregnant females were fed pellets containing pDNA (100 µg) daily, starting at day 5 after established pregnancy. Females and foeti were killed at days 7 and 14 of gestation, and pups at the time of weaning. Genomic DNA was analyzed by PCR followed by Southern blot and real-time PCR. A 201 bp target sequence was detected in mesenteric lymph nodes, spleen, liver and pancreas from growing rats 2 h p.f. At 6 h, target DNA was detectable in the kidneys, and at three days p.f. in the liver. Target DNA was not detected in samples from pregnant rats, their foeti or pups. In conclusion, low level of feed introduced DNA could be transiently detected in organs of young, growing rats. However, indications of increased DNA uptake levels in the GIT of growing rats were not found.

Artigo completo disponível em http://www.scirp.org/journal/PaperInformation.aspx?paperID=5447#.VICqE_ldW0c

Spisák, S.; Solymosi, N.; Ittzés, P.; Bodor, A.; Kondor, D.; Vattay, G.; Barták, B.; Sipos, F.; Galamb, O.; Tulassay, Z.; Szállási, Z.; Rasmussen, S.; Sicheritz-Ponten, T.; Brunak, S.;

Molnár, B.; Csabai, I. 2013. Complete genes may pass from food to human blood. *PLOS ONE*, 8(7): e69805. doi:10.1371/journal.pone.0069805.

Our bloodstream is considered to be an environment well separated from the outside world and the digestive tract. According to the standard paradigm large macromolecules consumed with food cannot pass directly to the circulatory system. During digestion proteins and DNA are thought to be degraded into small constituents, amino acids and nucleic acids, respectively, and then absorbed by a complex active process and distributed to various parts of the body through the circulation system. Here, based on the analysis of over 1000 human samples from four independent studies, we report evidence that meal-derived DNA fragments which are large enough to carry complete genes can avoid degradation and through an unknown mechanism enter the human circulation system. In one of the blood samples the relative concentration of plant DNA is higher than the human DNA. The plant DNA concentration shows a surprisingly precise log-normal distribution in the plasma samples while non-plasma (cord blood) control sample was found to be free of plant DNA.

Artigo completo disponível em http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0069805

4.2 Possibilidade de transferência horizontal de (trans)genes em células de mamíferos ou em micro-organismos simbiontes (bactérias do sistema digestivo e/ou da cavidade bucal em especial)

Um aspecto-chave em relação à estabilidade do DNA transgênico, depois de submetido a pressões do sistema digestivo, diz respeito à possibilidade subsequente de esse material genético vir a ser envolvido em processos de transferência horizontal em células e/ou bactérias simbiontes, integradas ao organismo que consumiu aquele DNA. Recentemente, tem crescido de forma expressiva o número de autores que consideram bactérias simbiontes do ser humano como fundamentais para diferentes funções relacionadas ao bem estar das pessoas, atuando como espécie de “órgão” distinto. De fato, há cerca de $10E^{17}$ bactérias num corpo humano. Trata-se de algo que supera de 10 a 100 vezes as estimativas do número de células humanas. Apenas no sistema digestivo essas bactérias compreendem mais de 500 espécies, das quais parte significativa se revela competente para realização de transferência horizontal de genes.

Os artigos a seguir discutem a importância desses mecanismos biológicos, sua complexidade e sua dependência de fatores, internos e externos, ainda não completamente conhecidos. Também destacam dificuldades de identificação e limites de detecção relacionados a tais eventos. A expressão "a ausência de evidência não significa evidência da ausência", criada por Carl Sagan, se aplica de forma clara ao tema dos riscos em processos afetados por mecanismos de THG.

Grillot-Courvalin, C.; Goussand, S.; Huetz, F.; Ojcius, D.; Courvalin, P. 1998. Functional gene transfer from intracellular bacteria to mammalian cells. *Nature Biotech*, 16: 862-66.

We provide evidence of direct transfer of functional DNA from bacteria to mammalian cells. An Escherichia coli K12 diaminopimelate auxotroph made invasive by cloning the invasin gene from Yersinia pseudotuberculosis transfers DNA after simple co-incubation, into a variety of mammalian cell lines. Transfer efficiency was enhanced in some cells by coexpression of the gene for listeriolysin from Listeria monocytogenes. Expression of the acquired genes occurs in both dividing and quiescent cells. The only requirement for bacteria to transfer genetic material into nonprofessional phagocytic cells and macrophages is the ability to invade the host cell.

http://www.ncbi.nlm.nih.gov/pubmed/9743121

Ho, M-W.; Traavik, T.; Olsvik, O.; Tappeser, B.; Howard, C.; Weizsacker, C.; McGavin, G. 1998. Gene technology and gene ecology of infectious diseases. *Microbial Ecology in Health and Disease*, 10: 33-59.

According to the 1996 WHO Report, the world is heading for a major crisis in public health as outbreaks of new and re-emerging infectious diseases are striking at increasing frequencies within the past 10 to 15 years. The current strains of pathogens are moreover, resistant to known treatments; some strains being resistant to all or nearly all drugs and antibiotics. Horizontal gene transfer is now generally recognized to be responsible for the evolution of virulence and the spread of drug and antibiotic resistances. Many pathogens have crossed species barriers, having acquired genes from phylogenetically distant species that are involved in their ability to cause diseases. Recent findings document the extremely wide scope of horizontal gene transfer and the extensive recombination between genetic material from unrelated species that have contributed to the emergence of virulence and antibiotic resistances. The past 15 years coincide with the development of genetic engineering biotechnology on a commercial scale. Genetic engineering depends on designing vectors for cloning and transferring genes and involves artificially recombining and manipulating genes from unrelated species and their viral pathogens, thereby enhancing the probability for horizontal gene transfer and recombination. The urgent question which needs to be addressed is the extent to which genetic engineering biotechnology, by facilitating horizontal gene transfer and recombination, is contributing to the resurgence of infectious, drug-resistant diseases, and will continue to do so if allowed to proceed unchecked. An enquiry into the possible contribution of genetic engineering biotechnology to the etiology of infectious diseases is all the more pressing in the light of other relevant recent findings indicating that microorganisms genetically engineered for 'contained use' may not be effectively contained. Thus, biologically 'crippled' strains of bacteria can survive in the environment to exchange genes with other species; DNA released from cells is not readily broken down in the environment, thereby retaining the ability to transform organisms; some viral DNA can be more infectious than the virus itself; and routine chemical treatments for inactivating pathogenic microorganisms and viruses, before they are discharged into the environment, may be ineffective, leaving a substantial percentage of pathogens in an active infectious state. The combination of the different kinds of evidence is sufficiently compelling, especially in view of the precautionary principle, to warrant, at the very least, an independent public enquiry into genetic engineering biotechnology and the etiology of infectious diseases.

Artigo complete disponível em http://www.microbecolhealthdis.net/index.php/mehd/article/view/7823

Mercer, D.; Scott, K.; Bruce-Johnson, W.; Glover, L.; Flint, H. 1999. Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology*, p. 6–10.

Competitive PCR was used to monitor the survival of a 520-bp DNA target sequence from a recombinant plasmid, pVACMC1, after admixture of the plasmid with freshly sampled human saliva. The fraction of the target remaining amplifiable ranged from 40 to 65% after 10 min of exposure to saliva samples from five subjects and from 6 to 25% after 60 min of exposure. pVACMC1 plasmid DNA that had been exposed to degradation by fresh saliva was capable of transforming naturally competent Streptococcus gordonii DL1 to erythromycin resistance, although transforming activity decreased rapidly, with a half-life of approximately 50 s. S. gordonii DL1 transformants were obtained in the presence of filter-sterilized saliva and a 1-microg/ml final concentration of pVACMC1 DNA. Addition of filter-sterilized saliva instead of heat-inactivated horse serum to S. gordonii DL1 cells induced competence, although with slightly lower efficiency. These findings indicate that DNA released from bacteria or food sources within the mouth has the potential to transform naturally competent oral bacteria. However, further investigations are needed to establish whether transformation of oral bacteria can occur at significant frequencies in vivo.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC90975/

Duggan, P.; Chambers, P.; Heritage, J.; Forbes, J. 2000. Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiology Letters*, 191, 71-77.

To assess the likelihood that the bla gene present in a transgenic maize line may transfer from plant material to the microflora associated with animal feeds, we have examined the survival of free DNA in maize silage effluent, ovine rumen fluid and ovine saliva. Plasmid DNA that had previously been exposed to freshly sampled ovine saliva was capable of transforming competent Escherichia coli cells to ampicillin resistance even after 24 h, implying that DNA released from the diet could provide a source of transforming DNA in the oral cavity of sheep. Although target DNA sequences could be amplified by polymerase chain reaction from plasmid DNA after a 30-min incubation in silage effluent and rumen contents, only short term biological activity, lasting less than 1 min, was observed in these environments, as shown by transformation to antibiotic resistance. These experiments were performed under in vitro conditions; therefore further studies are needed to elucidate the biological significance of free DNA in the rumen and oral cavities of sheep and in silage effluent.

http://www.ncbi.nlm.nih.gov/pubmed/11004402

Kunik, T.; Tzfira, T.; Kapulnik, Y.; Gafni, Y.; Dingwall, C.; Citovsky, V. 2001. Genetic transformation of HeLa cells by *Agrobacterium*. *PNAS*, 98 (4): 1871-6.

Agrobacterium tumefaciens is a soil phytopathogen that elicits neoplastic growths on the host plant species. In nature, however, *Agrobacterium* also may encounter organisms belonging to other kingdoms such as insects and animals that feed on the infected plants. Can *Agrobacterium*, then, also infect animal cells? Here, we report that *Agrobacterium* attaches to and genetically transforms several types of human cells. In stably transformed HeLa cells, the integration event occurred at the right border of the tumor-inducing plasmid's transferred-DNA (T-DNA), suggesting bona fide T-DNA transfer and lending support to the notion that *Agrobacterium* transforms human cells by a mechanism similar to that which it uses for transformation of plants cells. Collectively, our results suggest that *Agrobacterium* can transport its T-DNA to human cells and integrate it into their genome.

Artigo completo disponível em http://www.pnas.org/content/98/4/1871.full.pdf+html

Mercer, D.; Scott, K.; Melville, C.; Glover, L.; Flint, H. 2001. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. *FEMS Microbiol Lett*, 200 (2): 163-7.

Transformation of Streptococcus gordonii DL1 by free DNA was studied in human saliva. Competent S. gordonii could be transformed in vitro with plasmid DNA that had been taken into the human mouth. Transformation also occurred with a plasmid that cannot replicate in S. gordonii, but that has a region of chromosomal homology, by integration into the bacterial chromosome, although linearised plasmid DNA gave no transformants. Linear chromosomal DNA fragments did however transform S. gordonii/Tn916 efficiently in saliva when regions of homology with the recipient chromosome flanked the marker gene. These findings are discussed in relation to the potential for acquisition of DNA sequences, including genetically modifiedDNA, by gut and oral bacteria.

http://www.ncbi.nlm.nih.gov/pubmed/11425469

Duggan, P.; Chambers, P.; Heritage, J.; Forbes, J. 2003. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. *British Journal of Nutrition*, 89: 159-66.

The polymerase chain reaction (PCR) technique was used to investigate the fate of a transgene in the rumen of sheep fed silage and maize grains from an insect-resistant maize line. A 1914-bp DNA fragment containing the entire coding region of the synthetic cryIA(b) gene was still amplifiable from rumen fluid sampled 5 h after feeding maize grains. The same target sequence, however, could not be amplified from rumen fluid sampled from sheep fed silage prepared from the genetically modified maize line. PCR amplification of a shorter (211-bp), yet still highly specific, target sequence was possible with rumen fluid sampled up to 3 and 24 h after feeding silage and maize grains, respectively. These findings indicate that intact transgenes from silage are unlikely to survive significantly in the rumen since a DNA sequence 211-bp long is very unlikely to transmit genetic information. By contrast, DNA in maize grains persists for a significant time and may, therefore, provide a source of transforming DNA in the rumen. In addition, we have examined the biological activity of plasmid DNA that had previously been exposed to the ovine oral cavity. Plasmid extracted from saliva sampled after incubation for 8 min was still capable of transforming competent Escherichia coli to kanamycin resistance, implying that DNA released from the diet within the mouth may retain sufficient biological activity for the transformation of competent oral bacteria.

Artigo completo disponível em http://www.mednat.org/alimentazione/Duggan_GMO_Mais.pdf

COM – Heritage, J. 2004. The fate of transgenes in the human gut. *Nat Biotechnol*, 22(2): 170-172.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/14755289

REV – Smets, B.; Barkay, T. 2005. Horizontal gene transfer: perspectives at a crossroads of scientific disciplines. *Nature Reviews Microbiology*, 3: 675-678.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/16145755

Rizzi, A.; Raddadi, N.; Sorlini, C.; Nordgård, L.; Nielsen, K.; Daffonchio, D. 2012. The stability and degradation of dietary DNA in the gastrointestinal tract of mammals: implications for horizontal gene transfer and the biosafety of GMOs. *Critical Reviews in Food Science and Nutrition*, 52 (2) 142-161 (DOI: 10.1080/10408398.2010.499480).

The fate of dietary DNA in the gastrointestinal tract (GIT) of animals has gained renewed interest after the commercial introduction of genetically modified organisms (GMO). Among the concerns regarding GM food, are the possible consequences of horizontal gene transfer (HGT) of recombinant dietary DNA to bacteria or animal cells. The exposure of the GIT to dietary DNA is related to the extent of food processing, food composition, and to the level of intake. Animal feeding studies have demonstrated that a minor amount of fragmented dietary DNA may resist the digestive process. Mammals have been shown to take up dietary DNA from the GIT, but stable integration and expression of internalized DNA has not been demonstrated. Despite the ability of several bacterial species to acquire external DNA by natural transformation, in vivo transfer of dietary DNA to bacteria in the intestine has not been detected in the few experimental studies conducted so far. However, major methodological limitations and knowledge gaps of the mechanistic aspects of HGT calls for methodological improvements and further studies to understand the fate of various types of dietary DNA in the GIT.

http://www.ncbi.nlm.nih.gov/pubmed/22059960

Nordgård, L.; Brusetti, L.; Raddadi, N.; Traavik, T.; Averhoff, B.; Nielsen, K. 2012. An investigation of horizontal transfer of feed introduced DNA to the aerobic microbiota of the gastrointestinal tract of rats. *BMC Research Notes*, 5:170.

Background: Horizontal gene transfer through natural transformation of members of the microbiota of the lower gastrointestinal tract (GIT) of mammals has not yet been described. Insufficient DNA sequence similarity for homologous recombination to occur has been identified as the major barrier to interspecies transfer of chromosomal DNA in bacteria. In this study we determined if regions of high DNA similarity between the genomes of the indigenous bacteria in the GIT of rats and feed introduced DNA could lead to homologous recombination and acquisition of antibiotic resistance genes.
Results: Plasmid DNA with two resistance genes (*npt*I and *aad*A) and regions of high DNA similarity to 16S rRNA and 23S rRNA genes present in a broad range of bacterial species present in the GIT, were constructed and added to standard rat feed. Six rats, with a normal microbiota, were fed DNA containing pellets daily over four days before sampling of the microbiota from the different GI compartments (stomach, small intestine, cecum and colon). In addition, two rats were

included as negative controls. Antibiotic resistant colonies growing on selective media were screened for recombination with feed introduced DNA by PCR targeting unique sites in the putatively recombined regions. No transformants were identified among 441 tested isolates.
Conclusions: The analyses showed that extensive ingestion of DNA (100 µg plasmid) per day did not lead to increased proportions of kanamycin resistant bacteria, nor did it produce detectable transformants among the aerobic microbiota examined for 6 rats (detection limit < 1 transformant per 1,1 × 10^8cultured bacteria). The key methodological challenges to HGT detection in animal feedings trials are identified and discussed. This study is consistent with other studies suggesting natural transformation is not detectable in the GIT of mammals.

Artigo completo disponível em http://www.biomedcentral.com/content/pdf/1756-0500-5-170.pdf

Oraby, H.; Kandil, M.; Hassan, A.; Al-Sharawi, H. 2014. Addressing the issue of horizontal gene transfer from a diet containing genetically modified components into rat tissues. *African Journal of Biotechnology*, 13(48), 4410-4418.

Genetically modified (GM) food crops are considered to have the potential of providing food security especially in developing countries. Scientists have raised concern over the hazards associated with the consumption of genetically modified organisms (GMOs). One of these hazards, which have great controversy reports, is the possible horizontal gene transfer from GM-food or feed to human or animal tissues. Many researches were conducted to investigate the presence of some transgenic sequences in animal tissues fed on GM- crops. Many of the inserted genes in the GM-crops are under the control of the promoter of the Cauliflower mosaic virus (CaMVP35S) and produce insecticidal proteins. Health hazards are suggested to accompany the ingestion of this promoter. CaMVP35S can function in a wide range of organisms (plants and animals). It has also been demonstrated that the CaMV-P35S promoter sequence can convert an adjacent tissue- and organ-specific gene promoter into a globally active promoter. The present work was conducted to evaluate the possibility of horizontal gene transfer from a diet containing DNA segments from Cauliflower mosaic virus -35S promoter (CaMVP-35S) to the cells of different organs of rats fed for three months on diets containing genetically modified components. Analysis of the results revealed that: 1) ingested fragments from the CaMV-35S promoter incorporated into blood, liver, and brain tissues of experimental rats, 2) The total mean of transfer of GM target sequences increased significantly by increasing the feeding durations, and 3) The affinity of different transgenic fragments from the ingested GM-diet, to be incorporated into the different tissues of rats varied from one target sequence to the other.

Artigo completo disponível em http://www.academicjournals.org/article/article1417172424_Oraby%20et%20al.pdf

4.3 Alterações não desejadas da expressão gênica por meio de pequenos RNA não codificadores (ncRNA/sRNAs)

Em algumas PGMs, o transgene inserido não conduz à produção de uma proteína, mas tão somente a transcritos de pequenos RNA não codificadores. Por meio desses transcritos, as empresas de biotecnologia buscam novos produtos. Entre estes incluem-se habilidades para inativar/suprimir a expressão de genes envolvidos em vias fisiológicas cruciais para determinados insetos pragas ou para evitar a replicação de patógenos relevantes em plantas cultivadas. O risco nesse caso associa-se ao fato de que, uma vez sintetizadas nas plantas transgênicas, essas moléculas serão também consumidas pelos seres humanos e/ou pelos animais destinados às cadeias alimentares.

Esse é o caso do feijão da Embrapa e outros eventos apresentados como desprovidos de riscos (para a saúde humana e o meio ambiente) pelo fato de não produzirem proteínas recombinantes. Descobertas recentes apontando funções biológicas para pequenos RNA não codificadores, associados a regulação gênica, abriram novos campos de debates.

De fato, a síntese de alguns tipos de ncRNA parece acompanhada pela formação (não desejada/controlada) de outras pequenas moléculas de RNA (em especial no caso dos dsRNA) – que também serão consumidas por organismos alvo e não alvo. O importante nesse ponto é que os dsRNA constituem estruturas biológicas de elevada estabilidade, tendentes a permanecer ativos diante dos principais mecanismos de silenciamento gênico (RNAi, PTGS e TGS). Além disso, os pequenos RNAs não codificadores envolvidos nesses mecanismos de regulação gênica (siRNA em especial) podem ter ações inespecíficas (*off-target*), multiplicando as possibilidades de efeitos não desejados e potencialmente perigosos para os organismos.

Em paralelo, como na maior parte dos efeitos que envolvem o epigenoma, o silenciamento gênico obtido por meio dos dsRNA será transmissível de geração em geração (em especial o de RNAi). Ressalta-se, assim, a importância de avaliações robustas e detalhadas, aplicadas à análise de riscos potenciais decorrentes do consumo dessas PGMs.

Parcela significativa da comunidade científica considera que o conhecimento disponível é insuficiente para compreensão do modo de ação das moléculas de dsRNA e seus efeitos sobre espécies alvo e não alvo[66]. O uso seguro dessas tecnologias em escala comercial exigirá avanços prévios da ciência básica, seguidos de testes aplicados, como condição para a massificação de tecnologias. A escassez de conhecimentos sobre riscos associados ao uso comercial de RNA não codificadores, sobre a saúde pública e o meio ambiente[67], é ilustrada nos artigos a seguir. Os documentos selecionados reúnem incertezas e lacunas de conhecimento sobre riscos e benefícios potenciais dessas tecnologias e seus desdobramentos, notadamente no rumo das terapias gênicas.

Elbashir, S.; Harborth, J.; Lendeckel, W.; Yalcin, A.; Weber, K.; Tuschl, T. 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, 411: 494–8.

RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. The mediators of sequence-specific messenger RNA degradation are 21- and 22-nucleotide small interfering RNAs (siRNAs) generated by ribonuclease III cleavage from longer dsRNAs. Here we show that 21-nucleotide siRNA duplexes specifically suppress expression of endogenous and heterologous genes in different mammalian cell lines, including human embryonic kidney (293) and HeLa cells. Therefore, 21-nucleotide siRNA duplexes provide a new tool for studying gene function in mammalian cells and may eventually be used as gene-specific therapeutics.

Artigo completo disponível em http://www.nature.com/nature/journal/v411/n6836/full/411494a0.html%3Ffree%3D2

Qiu, S.; Adema, C.; Lane, T. 2005. A computational study of off-target effects of RNA interference. *Nucleic Acids Research*, 33: 1834–1847.

RNA interference (RNAi) is an intracellular mechanism for post-transcriptional gene silencing that is frequently used to study gene function. RNAi is initiated by short interfering RNA (siRNA) of approximately 21 nt in length, either generated from the double-stranded RNA (dsRNA) by using the enzyme Dicer or introduced experimentally. Following association with an RNAi silencing complex, siRNA targets mRNA transcripts that have sequence identity for destruction. A phenotype resulting from this knockdown of expression may inform about the function of the targeted gene. However, 'off-target effects' compromise the specificity of RNAi if sequence identity between siRNA and random mRNA transcripts causes RNAi to knockdown expression of non-targeted genes. The complete off-target effects must be investigated systematically on each gene in a genome by adjusting a group of parameters, which is too expensive to conduct experimentally and motivates a study in silico. This computational study examined the potential for off-target effects of RNAi, employing the genome and transcriptome sequence data of Homo sapiens, Caenorhabditis elegans and Schizosaccharomyces pombe. The chance for RNAi off-target effects proved considerable, ranging from 5 to 80% for each of the organisms, when using as parameter

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[66] Mais informações sobre o tema do silenciamento gênico por mecanismos epigenéticos estão disponíveis no item 1.2 da Parte 1.

[67] No item 2.3 da Parte 2 encontram-se informações complementares sobre fragilidades de tecnologias que recorrem ao uso de RNA não codificador, em especial por meio de silenciamento gênico pós-transcricional (PTGS). Esse é o caso de plantas geneticamente modificadas para resistir a vírus.

the exact identity between any possible siRNA sequences (arbitrary length ranging from 17 to 28 nt) derived from a dsRNA (range 100-400 nt) representing the coding sequences of target genes and all other siRNAs within the genome. Remarkably, high-sequence specificity and low probability for off-target reactivity were optimally balanced for siRNA of 21 nt, the length observed mostly in vivo. The chance for off-target RNAi increased (although not always significantly) with greater length of the initial dsRNA sequence, inclusion into the analysis of available untranslated region sequences and allowing for mismatches between siRNA and target sequences. siRNA sequences from within 100 nt of the 5' termini of coding sequences had low chances for off-target reactivity. This may be owing to coding constraints for signal peptide-encoding regions of genes relative to regions that encode for mature proteins. Off-target distribution varied along the chromosomes of C.elegans, apparently owing to the use of more unique sequences in gene-dense regions. Finally, biological and thermodynamical descriptors of effective siRNA reduced the number of potential siRNAs compared with those identified by sequence identity alone, but off-target RNAi remained likely, with an off-target error rate of approximately 10%. These results also suggest a direction for future in vivo studies that could both help in calibrating true off-target rates in living organisms and also in contributing evidence toward the debate of whether siRNA efficacy is correlated with, or independent of, the target molecule. In summary, off-target effects present a real but not prohibitive concern that should be considered for RNAi experiments.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1072799/

Kulkarni, M.; Booker, M.; Silver, S.; Friedman, A.; Hong, P.; Perrimon, N.; Mathey-Prevot, B. 2006. Evidence of off-target effects associated with long dsRNAs in *Drosophila melanogaster* cell-based assays. *Nature Methods*, 3: 833–838.

To evaluate the specificity of long dsRNAs used in high-throughput RNA interference (RNAi) screens performed at the Drosophila RNAi Screening Center (DRSC), we performed a global analysis of their activity in 30 genome-wide screens completed at our facility. Notably, our analysis predicts that dsRNAs containing > or = 19-nucleotide perfect matches identified in silico to unintended targets may contribute to a significant false positive error rate arising from off-target effects. We confirmed experimentally that such sequences in dsRNAs lead to false positives and to efficient knockdown of a cross-hybridizing transcript, raising a cautionary note about interpreting results based on the use of a single dsRNA per gene. Although a full appreciation of all causes of false positive errors remains to be determined, we suggest simple guidelines to help ensure high-quality information from RNAi high-throughput screens.

Artigo completo disponível em http://www.flyrnai.org/supplement/16964256.pdf

Fedorov, Y.; Anderson, E.; Birmingham, A.; Reynolds, A.; Karpilow, J.; Robinson, K.; Leake, D.; Marshall, W.; Khvorova, A. 2006. Off-target effects by siRNA can induce toxic phenotype. *RNA*, 12(7): 1188–1196.

Although recent microarray studies have provided evidence of RNA interference (RNAi)-mediated off-target gene modulation, little is known about whether these changes induce observable phenotypic outcomes. Here we show that a fraction of randomly selected small inhibitory RNAs (siRNAs) can induce changes in cell viability in a target-independent fashion. The observed toxicity requires an intact RNAi pathway and can be eliminated by the addition of chemical modifications that reduce off-target effects. Furthermore, an analysis of toxic and nontoxic duplexes identifies a strong correlation between the toxicity and the presence of a 4-base-pair motif (UGGC) in the RISC-entering strand of toxic siRNA. This article provides further evidence of siRNA-induced off-target effects generating a measurable phenotype and also provides an example of how such undesirable phenotypes can be mitigated by addition of chemical modifications to the siRNA.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1484448/

COM - Gordon, K.; Waterhouse, P. 2007. RNAi for insect-proof plants. *Nat Biotechnol*, 25:1231–2.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/17989682

Alemán, L.; Doench, J.; Sharp, P. 2007. Comparison of siRNA-induced off-target RNA and protein effects. *RNA*, 13: 385–395.

The downregulation of many mRNAs has been observed through bioinformatic analysis of microarray results following transfection of short interfering RNAs (siRNAs). Many of these mRNA changes are due to the interaction of the siRNA guide strand with partially complementary sites and thus are considered “off-target” effects. To examine the mRNA:siRNA interactions important for off-target effects, we generated a panel of mRNA:siRNA combinations containing single and double mismatches, bulges, and noncanonical base-pairing interactions in the 9th, 10th, and 11th positions of two siRNA binding sites located in the 3’ UTR of an integrated reporter gene. Approximately half of the mRNA:siRNA combinations containing mismatches in positions 9-11 result in a twofold or more mRNA decrease with varying degrees of protein knockdown. However, mRNA and protein analysis of the various mRNA:siRNA combinations reveals instances in which mRNA and protein levels do not correlate. Analysis of the resulting degradation products recovered from an imperfectly complementary siRNA interaction with an endogenous gene reveals a small fraction of products that map to the canonical siRNA cleavage site. Furthermore, downregulation of ARGONAUTE 2 (AGO2), the only AGO family protein known to catalyze canonical siRNA-mediated cleavage, did not significantly affect the degree of mRNA knockdown observed for one of the stably expressed reporters after transfection of an imperfectly complementary siRNA. These results indicate that although some degree of canonical siRNA cleavage can take place between a siRNA and an off-target transcript, most off-target mRNA reductions are likely attributable to AGO2-independent degradation processes.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1800510/

Hawkins, P.; Santoso, S.; Adams, C.; Anest, V.; Morris, K. 2009. Promoter targeted small RNAs induce long-term transcriptional gene silencing in human cells. *Nucleic Acids Research*, 37.

Small RNAs targeted to gene promoters in human cells can mediate transcriptional gene silencing (TGS) by directing silent state epigenetic modifications to targeted loci. Many mechanistic details of this process remain poorly defined, and the ability to stably modulate gene expression in this manner has not been explored. Here we describe the mechanisms of establishment and maintenance of long-term transcriptional silencing of the human ubiquitin C gene (*UbC*). Sustained targeting of the *UbC*promoter with a small RNA for a minimum of 3 days resulted in long-term silencing which correlated with an early increase in histone methylation and a later increase in DNA methylation at the targeted locus. Transcriptional silencing of *UbC* required the presence of a promoter-associated RNA. The establishment and maintenance of the TGS were shown to require distinct protein factors. Argonaute 1 (Ago1), DNA methyltransferase 3a (DNMT3a) and histone deacetylase 1 (HDAC1) were required for the initiation of silencing, and DNA methyltransferase 1 (DNMT1) was necessary for maintenance. Taken together the data presented here highlight the cellular pathway with which noncoding RNAs interact to epigenetically regulate gene expression in human cells.

Artigo completo disponível em http://nar.oxfordjournals.org/content/early/2009/03/20/nar.gkp127.full.pdf+html

REV – Jackson, A.; Linsley, P. 2010. Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. *Nature Reviews Drug Discovery*, 9: 57–67 (3).

Small interfering RNAs (siRNAs) are widely used to study gene function owing to the ease with which they silence target genes, and there is considerable interest in their potential for therapeutic applications. In a remarkably short time since their discovery, siRNAs have entered human clinical trials in various disease areas. However, rapid acceptance of the use of siRNAs has been accompanied by recognition of several hurdles for the technology, including a lack of specificity. Off-target activity can complicate the interpretation of phenotypic effects in gene-silencing experiments and can potentially lead to unwanted toxicities. Here, we describe the types of off-target effects of siRNAs and methods to mitigate them, to help enable effective application of this exciting technology.

http://www.ncbi.nlm.nih.gov/pubmed/20043028

REV – Huvenne, H.; Smagghe, G. 2010. Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. *J Insect Physiol*, 56(3):227-35. doi: 10.1016/j.jinsphys.2009.10.004.

RNA interference already proved its usefulness in functional genomic research on insects, but it also has considerable potential for the control of pest insects. For this purpose, the insect should be able to autonomously take up the dsRNA, for example through feeding and digestion in its midgut. In this review we bring together current knowledge on the uptake mechanisms of dsRNA in insects and the potential of RNAi to affect pest insects. At least two pathways for dsRNA uptake in insects are described: the transmembrane channel-mediated uptake mechanism based on Caenorhabditis elegans' SID-1 protein and an 'alternative' endocytosis-mediated uptake mechanism. In the second part of the review dsRNA feeding experiments on insects are brought together for the first time, highlighting the achievement of implementing RNAi in insect control with the first successful experiments in transgenic plants and the diversity of successfully tested insect orders/species and target genes. We conclude with points of discussion and concerns regarding further research on dsRNA uptake mechanisms and the promising application possibilities for RNAi in insect control.

http://www.ncbi.nlm.nih.gov/pubmed/19837076

REV – Davidson, B.; McCray, P. 2011. Current prospects for RNA interference–based therapies. *Nature Reviews Genetics*, 12: 329–340.

RNA interference (RNAi) is a powerful approach for reducing expression of endogenously expressed proteins. It is widely used for biological applications and is being harnessed to silence mRNAs encoding pathogenic proteins for therapy. Various methods - including delivering RNA oligonucleotides and expressing RNAi triggers from viral vectors - have been developed for successful RNAi in cell culture and in vivo. Recently, RNAi-based gene silencing approaches have been demonstrated in humans, and ongoing clinical trials hold promise for treating fatal disorders or providing alternatives to traditional small molecule therapies. Here we describe the broad range of approaches to achieve targeted gene silencing for therapy, discuss important considerations when developing RNAi triggers for use in humans, and review the current status of clinical trials.

http://www.ncbi.nlm.nih.gov/pubmed/21499294

COM – Hirschi, K. 2012. New foods for thought. *Trends Plant Sci*, 17:123–5.

Recent findings show that genetic material in plant foods may survive digestion, circulate through our bodies and modulate our gene expression. These findings could alter our understanding of nutrition, genetic regulation and open up new vistas for engineering foods.

http://www.ncbi.nlm.nih.gov/pubmed/22265093

Zhang, L.; Hou, D.; Chen, X.; Li, D.; Zhu, l.; Zhang, y.; Li, j.; Bian, Z.; Liang, X.; Cai, X.; Yin, Y.; Wang, C.; Zhang, T.; Zhu, D.; Zhang, D.; Xu, J.; Chen, Q.; Ba, Y.; Liu, J.; Wang, Q.; Chen, J.; Wang, J.; Wang, M.; Zhang, Q.; Zhang, J.; Zen, K.; Zhang, C-Y. 2012. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: Evidence of cross-kingdom regulation by microRNA. *Cell Research*, 22(1):107-26 (Erratum in: Cell Res, 22(1): 273-4).

Our previous studies have demonstrated that stable microRNAs (miRNAs) in mammalian serum and plasma are actively secreted from tissues and cells and can serve as a novel class of biomarkers for diseases, and act as signaling molecules in intercellular communication. Here, we report the surprising finding that exogenous plant miRNAs are present in the sera and tissues of various animals and that these exogenous plant miRNAs are primarily acquired orally, through food intake. MIR168a is abundant in rice and is one of the most highly enriched exogenous plant miRNAs in the sera of Chinese subjects. Functional studies in vitro and in vivo demonstrated that MIR168a could bind to the human/mouse low-density lipoprotein receptor adapter protein 1 (LDLRAP1) mRNA, inhibit LDLRAP1 expression in liver, and consequently decrease LDL removal from mouse plasma. These findings demonstrate that exogenous plant miRNAs in food can regulate the expression of target genes in mammals.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3351925/

REV - Heinemann, J.; Agapito-Tenfen, S.; Carman, J. 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. *Environment International*, Vol. 55: 43-55.

Changing the nature, kind and quantity of particular regulatory-RNA molecules through genetic engineering can create biosafety risks. While some genetically modified organisms (GMOs) are intended to produce new regulatory-RNA molecules, these may also arise in other GMOs not intended to express them. To characterise, assess and then mitigate the potential adverse effects arising from changes to RNA requires changing current approaches to food or environmental risk assessments of GMOs. We document risk assessment advice offered to government regulators in Australia, New Zealand and Brazil during official risk evaluations of GM plants for use as human food or for release into the environment (whether for field trials or commercial release), how the regulator considered those risks, and what that experience teaches us about the GMO risk assessment framework. We also suggest improvements to the process.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0160412013000494

REV - Lundgren, J.; Duan, J. 2013. RNAi-Based insecticidal crops: Potential effects on nontarget species. *BioScience*, 63(8):657-665.

The potential hazards posed by RNA interference (RNAi)—based pesticides and genetically modified crops to nontarget organisms include off-target gene silencing, silencing the target gene in unintended organisms, immune stimulation, and saturation of the RNAi machinery. Nontarget organisms will vary in their exposure to small RNAs produced by genetically modified crops, but exposure to insecticidal small RNAs will probably occur at a previously unrealized scale for many. Areas that warrant future work include the persistence of insecticidal small RNAs in the environment, describing crop-based food webs to understand those species that are most exposed, sequencing genomes for species to proactively understand those that may be affected by RNAi, and substantiating that laboratory toxicity testing can accurately predict the field-level effects of this technology. The costs and benefits of pesticidal RNA must be considered relative to current pest management options as pesticidal RNAs move from a theoretical approach to being used as a practical tool.

http://www.bioone.org/doi/abs/10.1525/bio.2013.63.8.8

Nunes, F.; Aleixo, A.; Barchuk, A.; Bomtorin, A.; Grozinger, C.; Simões, Z. 2013. Non-Target Effects of green fluorescent protein (GFP)-derived double-stranded RNA (dsRNA-GFP) used in honey bee RNA interference (RNAi) assays. *Insects*, *4*, 90-103; doi:10.3390/insects4010090.

RNA interference has been frequently applied to modulate gene function in organisms where the production and maintenance of mutants is challenging, as in our model of study, the honey bee, Apis mellifera. A green fluorescent protein (GFP)-derived double-stranded RNA (dsRNA-GFP) is currently commonly used as control in honey bee RNAi experiments, since its gene does not exist in the A. mellifera genome. Although dsRNA-GFP is not expected to trigger RNAi responses in treated bees, undesirable effects on gene expression, pigmentation or developmental timing are often observed. Here, we performed three independent experiments using microarrays to examine the effect of dsRNA-GFP treatment (introduced by feeding) on global gene expression patterns in developing worker bees. Our data revealed that the expression of nearly 1,400 genes was altered in response to dsRNA-GFP, representing around 10% of known honey bee genes. Expression changes appear to be the result of both direct off-target effects and indirect downstream secondary effects; indeed, there were several instances of sequence similarity between putative siRNAs generated from the dsRNA-GFP construct and genes whose expression levels were altered. In general, the affected genes are involved in important developmental and metabolic processes associated with RNA processing and transport, hormone metabolism, immunity, response to external stimulus and to stress. These results suggest that multiple dsRNA controls should be employed in RNAi studies in honey bees. Furthermore, any RNAi studies involving these genes affected by dsRNA-GFP in our studies should use a different dsRNA control.

Artigo completo disponível em http://www.mdpi.com/2075-4450/4/1/90

Alguns artigos examinam riscos imunológicos associados ao uso de tecnologias que conduzem à formação de moléculas de RNA não codificadores (pequenos RNA de interferência em especial – siRNA). De fato, alguns siRNA – pertencentes à maquinaria de RNA interferência – parecem ter papel-chave na regulação do sistema imunológico, em mamíferos.

Judge, A.; Sood, V.; Shaw, J.; Fang, D.; McClintock, K.; MacLachlan, I. 2005. Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA. *Nature Biotechnology*, 23: 457–462.

Short interfering RNAs (siRNAs) that mediate specific gene silencing through RNA interference (RNAi) are widely used to study gene function and are also being developed for therapeutic applications. Many nucleic acids, including double- (dsRNA) and single-stranded RNA (ssRNA), can stimulate innate cytokine responses in mammals. Despite this, few studies have questioned whether siRNA may have a similar effect on the immune system. This could significantly influence the *in vivo* application of siRNA owing to off-target effects and toxicities associated with immune stimulation. Here we report that synthetic siRNAs formulated in nonviral delivery vehicles can be potent inducers of interferons and inflammatory cytokines both *in vivo* in mice and *in vitro* in human blood. The immunostimulatory activity of formulated siRNAs and the associated toxicities are dependent on the nucleotide sequence. We have identified putative immunostimulatory motifs that have allowed the design of siRNAs that can mediate RNAi but induce minimal immune activation.

Artigo completo disponível em http://www.nature.com/nbt/journal/v23/n4/full/nbt1081.html

REV - Marques, J.; Williams, B. 2005. Activation of the mammalian immune system by siRNAs. *Nature Biotechnology*, 23, 1399-1405 doi:10.1038/nbt1161.

Inhibition of gene expression through RNA interference (RNAi) is emerging as a powerful experimental tool for gene function and target validation studies. The potential uses of this technology seem unlimited, extending to the prevention and therapy of human diseases. However, recent work demonstrating that there are unanticipated, different nonspecific effects associated with the use of small interfering RNAs in mammals has raised concerns about the safe use of RNAi in vivo. These nonspecific effects include activation of the immune system, potentially harming the individual. The application of screening assays for nonspecific activation of both innate and acquired immunity will be necessary for further development of RNAi as a therapeutic tool.

http://www.ncbi.nlm.nih.gov/pubmed/16273073

Robbins, M.; Judge, A.; MacLachlan, I. 2009. siRNA and innate immunity. *Oligonucleotides*, 19: 89–102.

Canonical small interfering RNA (siRNA) duplexes are potent activators of the mammalian innate immune system. The induction of innate immunity by siRNA is dependent on siRNA structure and sequence, method of delivery, and cell type. Synthetic siRNA in delivery vehicles that facilitate cellular uptake can induce high levels of inflammatory cytokines and interferons after systemic administration in mammals and in primary human blood cell cultures. This activation is predominantly mediated by immune cells, normally via a Toll-like receptor (TLR) pathway. The siRNA sequence dependency of these pathways varies with the type and location of the TLR involved. Alternatively nonimmune cell activation may also occur, typically resulting from siRNA interaction with cytoplasmic RNA sensors such as RIG1. As immune activation by siRNA-based drugs represents an undesirable side effect due to the considerable toxicities associated with excessive cytokine release in humans, understanding and abrogating this activity will be a critical component in the development of safe and effective therapeutics. This review describes the intracellular mechanisms of innate immune activation by siRNA, the design of appropriate sequences and chemical modification approaches, and suitable experimental methods for studying their effects, with a view toward reducing siRNA-mediated off-target effects.

http://www.ncbi.nlm.nih.gov/pubmed/19441890

Parte 5

Polêmicas científicas e críticas ao processo de análise do risco das plantas transgênicas

Em paralelo às críticas existentes sobre processos de análise do risco com plantas transgênicas, acumulam-se contradições em publicações científicas que observam, negam ou sugerem impactos negativos desses organismos geneticamente modificados – sobre o ambiente e saúde –, independentemente do campo da biologia considerado.

Em que pese a intensidade dos debates, chama atenção o fato de que autores de pesquisas que apontam problemas venham sendo alvo de campanhas difamatórias organizadas, em geral, por atores (pesquisadores ou não) comprometidos com o sucesso da biotecnologia e seus produtos.

Os referidos ataques, que avançam sobre aspectos pessoais, costumam partir de detalhes metodológicos secundários, fazendo pouco caso de situações em que os pontos de crítica já se haviam consolidado, constituindo opções metodológicas aceitas desde que incorporadas a estudos favoráveis às PGMs. Destacam-se, nessas situações, a adoção de argumentos compostos pela lógica distorcida de "dois pesos e duas medidas" por parte dos defensores da biotecnologia.

1 Críticas às "bases científicas" da avaliação do risco

Desde a introdução das plantas transgênicas na alimentação humana, (anos 1990), cresce e se consolida a desconfiança quanto a mecanismos e protocolos utilizados por instâncias reguladoras comprometidas com a avaliação de riscos em OGMs. Dentre os motivos destaca-se a coincidência de interpretação. Em todos os casos, as agências reguladoras utilizam estudos produzidos ou financiados pelas empresas de biotecnologia, para validar pressupostos de segurança biológica por elas reclamados. Mais do que isso, desprezam estudos que apontam conclusões opostas.

Há alguns anos, vários segmentos da sociedade civil organizada e pesquisadores independentes alertam a sociedade global para a falta de transparência e de rigor científico presente em mecanismos decisórios que, com base em análises de curto prazo, sustentam inexistência de riscos de longo prazo. Também chamam atenção para a fragilidade de métodos, insuficiência de amostras, incoerência de resultados e ocultação de dados necessários à verificação das decisões.

Além disso, parte significativa da comunidade científica tem manifestado divergências quanto à insuficiência e à inadequação de protocolos e abordagens utilizados por órgãos reguladores. Destacam-se, neste ponto, elementos quase insólitos, como o conceito de "equivalência substancial" e sua sobrevalorização, em detrimento de compromissos internacionais relacionados ao "princípio da precaução".

Os artigos referenciados a seguir mostram que as análises do risco devem manter ligação estrita com aspectos culturais e socioeconômicos dominantes nas comunidades que serão afetadas. Assim, decisões de estimular ou refrear o uso comercial de biotecnologias agrícolas sempre possuirão conotações políticas, com poder de filtro e juízos próprios de valoração a serem ponderados a partir dos conhecimentos e das incertezas científicas, bem como de suas implicações.

COM - Millstone, E.; Brunner, E.; Mayer, S. 1999. Beyond 'substantial equivalence'. *Nature*, Vol 401.

Showing that a genetically modified food is chemically similar to its natural counterpart is not adequate evidence that it is safe for human consumption. Whenever official approval for the introduction of genetically modified (GM) foods has been given in Europe or the United States, regulatory committees have invoked the concept of 'substantial equivalence'. This means that if a GM food can be characterized as substantially equivalent to its 'natural' antecedent, it can be assumed to pose no new health risks and hence to be acceptable for commercial use.

http://www.nature.com/nature/journal/v401/n6753/full/401525a0.html

Nodari, R.; Guerra, M. 2000. Implications of transgenics for environmental and agricultural sustainability. *História, Ciências, Saúde;* Manguinhos, 7(2): 481-491.

The potential risks of GMOs, their impact on human and animal health, and on the environment, as well as their socioeconomic effects, have generated a worldwide discussion which is far from drawing to a close for lack of sufficient scientific information. Part of this information supports risk-hypotheses previously put forward. Thus the presence of transgenic plant genes in other plants and in other organisms has been confirmed in several occasions. Therefore, gene dissemination to plants of the same species as well as to widely different species is already regarded as an actual risk. The principle of substantial equivalence has opened the way for the liberation of transgenic plants for commercial crops, despite short-term tests, which are quantitatively and qualitatively insufficient to certify that the foods deriving from those plants are healthy and safe. Thus, the adoption of the so-called precautionary principle (PP) has turned out to be the most adequate safety measure to date, or else until scientific data should be able to demonstrate the actual impact of transgenic plants on human and animal health, and on the environment.

Artigo completo disponível em http://www.scielo.br/scielo.php?pid=S0104-59702000000300016&script=sci_arttext

Zdunczyk, Z. 2001. In vivo experiments on the safety evaluation of GM components of feeds and foods. *Journal of Animal and Feed Sciences*, 10 (Supplement 1): 195-210.

During the last five years, the global area of transgenic crop (GM-genetically modified) cultivation increased 25-fold. About 98% of GM crops are grown in the USA, Argentina and Canada from where they are sent to many importers of soyabean and maize. The results of feeding experiments indicate that soyabean meal obtained from herbicide-tolerant lints and insect-resistant maize are substantially and nutritionally equivalent to their conventional lines. A higher content of insecticidal alpha -amylase inhibitors las well as lectins and alkaloids) may increase plants' resistance to insect attack, as well as the decrease nutritional value of seeds. Evaluation of the concordance of the chemical composition of transgenic and conventional crops (i.e., verification of substantial equivalence) is not sufficient for proving the safety of transgenic food. Sub-chronic in vivo experiments as well as comparison of nutritional equivalence of transgenic and conventional crops are advisable. Such actions are justified not only by the possibility of undesirable transgenic effects, but also by the consumer's right to explicit information on food safety. Without evaluation of nutritional equivalence, information on GM-food safety is much more deficient than existing knowledge on the quality of feeds used in animal nutrition.

http://serials.unibo.it/cgi-ser/start/it/spogli/df-s.tcl?prog_art=8768196&view=articoli

REV – Myhr, A.; Traavik, T. 2002. The precautionary principle: scientific uncertainty and omitted research in the context of GMO use and release. *JAGE (Journal of Agricultural and Environmental Ethics)*, 15: 73-86.

Commercialization of genetically modified organisms (GMOs) have sparked profound controversies concerning adequate approaches to risk regulation. Scientific uncertainty and ambiguity, omitted research areas, and lack of basic knowledge crucial to risk assessments have become apparent. The objective of this article is to discuss the policy and practical implementation of the Precautionary Principle. A major conclusion is that the void in scientific understanding concerning risks posed by secondary effects and the complexity of cause-effect relations warrant further research. Initiatives to approach the acceptance or rejection of a number of risk-associated hypotheses is badly needed. Further, since scientific advice plays a key role in GMO regulations, scientists have a responsibility to address and communicate uncertainty to policy makers and the public. Hence, the acceptance of uncertainty is not only a scientific issue, but is related to public policy and involves an ethical dimension.

Artigo completo disponível em https://www.cbd.int/doc/articles/2008/A-00637.pdf

Levidow, L.; Murphy, J.; Carr, S. 2007. Recasting "substantial equivalence": Transatlantic governance of GM food. *Science, Technology, and Human Values*; 32(1): 26–64.

When intense public controversy erupted around agricultural biotechnology in the late 1990s, critics found opportunities to challenge risk assessment criteria and test methods for genetically modified (GM) products. In relation to GM food, they criticized the concept of substantial equivalence, which European Union and United States regulators had adopted as the basis for a harmonized, science-based approach to risk assessment. Competing policy agendas framed scientific uncertainty in different ways. Substantial equivalence was contested and eventually recast to accommodate some criticisms. To explain how the concept changed, this article links two analytical perspectives. Regulatory-science perspectives illuminate how the scientification of politics and politicization of science led to shifts in the boundary between science and policy. Governance perspectives illuminate how the collective problem for policy was redefined to provide a new common ground for some stakeholders. Overall, substantial equivalence was recast to govern the social conflict and address legitimacy problems of regulatory procedures.

Artigo completo disponível em http://oro.open.ac.uk/6751/1/LLJMSC_SubstEquiv_STHV_07corr.pdf

Zolla, L.; Rinalducci, S.; Antonioli, P.; Righetti, P. 2008. Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. *Journal of Proteome Research*, 7(5): 1850-1861.

To improve the probability of detecting unintended side effects during maize gene manipulations by bombardment, proteomics was used as an analytical tool complementary to the existing safety assessment techniques. Since seed proteome is highly dynamic, depending on the species variability and environmental influence, we analyzed the proteomic profiles of one transgenic maize variety (event MON 810) in two subsequent generations (T05 and T06) with their respective isogenic controls (WT05 and WT06). Thus, by comparing the proteomic profiles of WT05 with WT06 we could determine the environmental effects, while the comparison between WT06 and T06 seeds from plants grown under controlled conditions enabled us to investigate the effects of DNA manipulation. Finally, by comparison of T05 with T06 seed proteomes, it was possible to get some indications about similarities and differences between the adaptations of transgenic and isogenic plants to the same strictly controlled growth environment. Approximately 100 total proteins resulted differentially modulated in the expression level as a consequence of the environmental influence (WT06 vs WT05), whereas 43 proteins resulted up- or down-regulated in transgenic seeds with respect to their controls (T06 vs WT06), which could be specifically related to the insertion of a single gene into a maize genome by particle bombardment. Transgenic seeds responded differentially to the same environment as compared to their respective isogenic controls, as a result of the genome rearrangement derived from gene insertion. To conclude, an exhaustive differential proteomic analysis allows to determine similarities and differences between traditional food and new products (substantial equivalence), and a case-by-case assessment of the new food should be carried out in order to have a wide knowledge of its features.

Artigo completo disponível em https://www.ufpe.br/biolmol/Tec-mol-biol/papers-TMB-2010/proteomica-Zolla_et_al-2008.pdf

Myhr, A. 2010. The challenge of scientific uncertainty and disunity in risk assessment and management of GM crops. *Environmental Values*, 19: 7-31. Doi: 10.3197/096327110X485365.

The controversy over commercial releases of genetically modified (GM) crops demonstrates that there is a need for new approaches that are more broadly based, transparent and able to acknowledge the uncertainties involved. This article investigates whether new forms of knowledge production as prescribed in the concept of post-normal science can improve risk governance of GM crops. The GM science review carried out in the UK in 2003 serves as a case study and the focus is on how scientific uncertainty and public concern was taken into account. Some recommendations are advanced for assessing scientific uncertainty, for accommodating scientific disputes and for integrating stakeholders' interests and perspectives in relations to GM crops.

http://www.erica.demon.co.uk/EV/EV1902.html

Myhr, A. 2010. A precautionary approach to genetically modified organisms: Challenges and implications for policy and science. *Journal of Agricultural and Environmental Ethics*. DOI:10.1007/s10806-010-9234-x.

The commercial introduction of genetically modified organisms (GMOs) has revealed a broad range of views among scientists and other stakeholders on perspectives of genetic engineering (GE) and if and how GMOs should be regulated. Within this controversy, the precautionary principle has become a contentious issue with high support from skeptical groups but resisted by GMO advocates. How to handle lack of scientific understanding and scientific disagreement are core issues within these debates. This article examines some of the key issues affecting precaution as a legal standard and as an approach to the use of science in decision-making processes. It is pointed out that there is a need for reflection over the level of scientific evidence required for applying the precautionary principle as well as who should have the burden of proof when there are uncertainties. Further, an awareness of the broader scientific uncertainties found in GMO risk assessment implies that a precautionary approach must be elaborated: both for acknowledging uncertainties and for identification of scientific responses. Since precaution is an important issue within the sustainable development framework, it is suggested that sustainability can provide a normative standard that can help to reveal the influence and negotiate the importance of the various forms of uncertainty. Wise management of uncertainties and inclusion of normative aspects in risk assessment and management may help to ensure sustainable and socially robust GMO innovations at present and in the future.

Artigo completo disponível em http://bch.cbd.int/database/record.shtml?documentid=101951

REV - Heinemann, J.; Kurenbach, B.; Quist, D. 2011. Molecular profiling — a tool for addressing emerging gaps in the comparative risk assessment of GMOs. *Environment International*, 37, 1285–1293.

Assessing the risks of genetically modified organisms (GMOs) is required by both international agreement and domestic legislation. Many view the use of the "omics" tools for profiling classes of molecules as useful in risk assessment, but no consensus has formed on the need or value of these techniques for assessing the risks of all GMOs. In this and many other cases, experts support case-by-case use of molecular profiling techniques for risk assessment. We review the latest research on the applicability and usefulness of molecular profiling techniques for GMO risk assessment. As more and more kinds of GMOs and traits are developed, broader use of molecular profiling in a risk assessment may be required to supplement the comparative approach to risk assessment. The literature-based discussions on the use of profiling appear to have settled on two findings: 1. profiling techniques are reliable and relevant, at least no less so than other techniques used in risk assessment; and 2. although not required routinely, regulators should be aware of when they are needed. The dismissal of routine molecular profiling may be confusing to regulators who then lack guidance on when molecular profiling might be worthwhile. Molecular profiling is an important way to increase confidence in risk assessments if the profiles are properly designed to address relevant risks and are applied at the correct stage of the assessment.

http://www.ncbi.nlm.nih.gov/pubmed/21624662

Wickson, F.; Wynne, B. 2012. Ethics of science for policy in the environmental governance of biotechnology: MON810 maize in Europe. *Ethics, Policy and Environment*; 15(3): 321-340.

This paper discusses entanglements of science and ethics in the regulation of genetically modified crops. Using the 2009 German ban of genetically modified maize MON810 and debates concerning the quality of science cited to support it, the paper highlights how values are tacitly embedded in science for policy and how ethical questions permeate the way this science is developed, quality-controlled, and given authority in the European regulation of biotechnology. We argue that a lack of recognition and inadequate treatment of such value-commitments influencing science, and through this policy, impinges upon and weakens the ethical standards involved. This has particular significance as Europe debates genetically modified crop legislative reform.

http://philpapers.org/rec/WICEOS

COM - Wickson, F. 2014. Environmental protection goals, policy & publics in the European regulation of GMOs. *Ecological Economics*, 108, 269–273.

One of the most divisive debates in modern agriculture concerns the use of genetically modified organisms (GMOs). In Europe, the policy debate over GMOs has been met with a persistent attempt to retreat into "sound science" as a potential unifying force. However, environmental risk assessment as an aid to regulatory decision-making is inherently entangled with questions of environmental ethics. This is particularly manifested in the setting of environmental protection goals. For the risk assessment of GMOs, the European Food Safety Authority has presented an inconsistent position on environmental protection goals. There is, however, an emerging trend for biodiversity conservation to be enfolded within an ecosystem services frame, and for ecosystem services to be reduced to biological terms. How environmental protection goals are understood, articulated and used to define risk assessment and shape regulatory decision-making is a significant factor in the entrenched debate over the regulation of GMOs in Europe. In negotiating this territory, I suggest that the attempt to enforce a strict divide between nature and culture or social and ecological systems in Europe's risk assessment of GMOs is emphatically counter-productive, for both robust science and considered ethical action.

http://www.sciencedirect.com/science/article/pii/S092180091400295X

Após quinze anos de comercialização de plantas Bt, persistem dúvidas básicas, tais como a quantidade de toxina Bt produzida em cada evento transgênico e razões de sua diferenciação/oscilação entre partes das plantas e períodos de desenvolvimento das culturas. Evidentemente essa incerteza gera situações de risco potenciais em relação à saúde humana e animal, bem como ao meio ambiente.

Saeglitz, C.; Bartsch, D.; Eber, A.; Gathmann, K.; Priesnitz, K.; Schuphan, I. 2006. Monitoring the Cry1Ab susceptibility of European corn borer in Germany. *Journal of Economic Entomology*, 99(5): 1768-1773.

The European corn borer, Ostrinia nubilalis (Hübner), is one of the most important insect pests in corn, Zea mays L. Transgenic corn cultivars expressing Bacillus thuringiensis (Bt) toxin provide a promising crop protection strategy against European corn borer; however, management is needed to avoid resistance development of the target pest species. The aim of this work was to establish the baseline susceptibility of different European corn borer populations in Germany to be able to forecast a possible development of resistance at an early stage. To standardize test procedures for future resistance management, the efficiency of Cry1Ab toxins from different suppliers and different production was assessed. Furthermore, two different test methods, surface treatment and the incorporation method, were compared with regard to their practicability and efficiency. Neither method provided significant differences in the baseline susceptibility of populations from different German regions. Overall, the data suggested little differentiation among German populations in terms of their susceptibility to Bt toxin and their genetic background. Future monitoring could therefore use a single European corn borer population as a representative for southwestern Germany. However, toxins from different suppliers and different production batches produced a vast range of LC50 values. Changes because of different toxin batches may be mistaken as a change in baseline susceptibility or even as the start of a resistance development. Thus, it is important throughout insect resistance management that the same toxin batches will be available for baseline susceptibility bioassays and for future tests.

Artigo completo disponível em http://www.biw.kuleuven.be/aee/clo/euwab_files/saeglitz2006.pdf

Székács, A.; Weiss G.; Quist, D.; Takács, E.; Darvas, B.; Meier, M.; Swain, T.; Hilbeck, A. 2011. Interlaboratory comparison of Cry1Ab toxin quantification in MON 810 maize by ezyme-immunoassay. *Food and Agricultural Immunology*, DOI:10.1080/09540105.2011.604773.

A laboratory ring trial was performed in four laboratories for determination of Cry1Ab toxin in leaf material of MON 810 maize using a standardised enzyme-linked immunoassay protocol. Statistical analysis was carried out by the ISO 5725-2 guidelines, sample standard deviation and standard error, within-laboratory and inter-laboratory SD and SE were calculated. Measured inter-laboratory average values were 12.5±4.0, 15.3±4.6 and 72.6±17.8 μg/g for three lyophilised samples, and 27.8±4.3 μg/g for a frozen sample, yet, Cry1Ab concentrations ranged 66.5–160.1% of the corresponding IA. Determined concentrations by in-house protocols were statistically not different in one laboratory and different in two laboratories from the corresponding values by the joint protocol. Results emphasise the importance of a standardised protocol among laboratories for comparable quantitative Cry1Ab toxin determination. However, even when using a standardised protocol, significant differences still occur among toxin concentrations detected in different laboratories, although with a smaller range of variation.

Artigo complete disponível em http://libra.msra.cn/Publication/57599316/interlaboratory-comparison-of-cry1ab-toxin-quantification-in-mon-810-maize-by-enzyme-immunoassay

2 Falta de rigor científico na avaliação do risco para a saúde

A falta de consenso científico sobre a ausência de riscos para a saúde, bem como problemas associados ao consumo diário de alimentos transgênicos, foi discutida na Parte 4 deste livro.

Como complementação, apresentamos a seguir conjunto de estudos críticos aos processos de análise do risco, tal como conduzidos pelos órgãos reguladores. Essa compilação incorpora fragilidades relacionadas a aspectos biológicos, questões socioeconômicas, jurídicas e éticas. Também inclui o monitoramento do consumo dessas PGMs, bem como as implicações de sua fragilidade (do monitoramento) para a saúde pública, em perspectiva de médio e longo prazo.

COM - Schubert, D. 2002. A different perspective on GM food. *Nature Biotechnology*, 20, 969.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/12355105

Freese, W.; Schubert, D. 2004. Safety testing and regulation of genetically engineered foods. *Biotechnology and Genetic Engineering Reviews*, Vol 21. 299-324.

The use of recombinant DNA techniques to engineer food crops with novel traits has aroused tremendous interest and concern throughout the world. Both the public and the scientific community are deeply divided on a host of issues raised by genetically engineered (GE) crops. Do they pose human health or environmental risks? Are they adequately regulated? Should foods containing them be labeled? Should society allow them to be patented? Are they relevant to the developing world? Science alone cannot and will not decide the many disputes that have arisen between and within nations over GE foods. As with the introduction of any powerful new technology, economic, cultural and ethical factors will also come into play. But science can help ground the debate, particularly in the contentious area of regulation.

Artigo completo disponível em http://www.mma.gov.br/estruturas/biosseguranca/_arquivos/71_28112008023818.pdf

Heinemann, J.; Sparrow, A.; Traavik, T. 2004. Is confidence in the monitoring of GE foods justified? *Trends in Biotechnology*, Vol.22 No.7.

Often the limits of detection of genetically engineered organisms (GEOs, LMOs, GMOs) determine what legislation sets as thresholds of allowable contamination of the human food chain with GEOs. Many countries have legislation that is triggered by certain thresholds of contamination. Importantly, international trade in food and animal feed is becoming increasingly vulnerable to interruptions caused by the ambiguity GEOs can create when shipments are monitored at the border. We examine the tools available for detection. Four key error-generating stages are identified with the aim of prompting a higher uniform standard of routine analysis at export and import points. Contamination of the New Zealand corn crop with GEOs is used as a case study for the application of monitoring tools and vulnerability to errors. These tools fail to meet emerging food safety requirements, but some improvements are in development.

http://www.ncbi.nlm.nih.gov/pubmed/15245904

Seetharam, S. 2010. Should the Bt brinjal controversy concern healthcare professionals and bio-ethicists? *Indian J Med Ethics*, 7 (1).

The Genetic Engineering Approval Committee's approval of Bt brinjal, the first genetically modified crop for human consumption in India, has sparked off protests across the country. This article questions the so-called benefits of GM crops and highlights some major concerns. These include: inadequately addressed health and environmental risks, inadequate safety guidelines, a lack of transparency in sharing test data, the implications to seed sovereignty of farmers and the lack of informed choice for consumers. Some concerns about field testing by Mahyco, the developer of Bt-brinjal, and the process of evaluation by GEAC remain unresolved. With inadequate information about the crop's long-term safety, a precautionary approach is advocated before national policy allows commercial release of the seeds. A fair process is also needed in the public consultations being proposed by the minister of state for environment and forests. In addition to issues of procedural justice, a basic ethical question remains: do humans have a right to dominate the land and make expendable those creatures that they deem "undesirable"?

http://www.ncbi.nlm.nih.gov/pubmed/20166287

2.1 Críticas da falta de rigor científico sobre avaliação do risco alergênico

Os estudos a seguir discutem fragilidades de protocolos de avaliação do risco alergênico, que em geral se limitam à comparação de homologia na sequência de aminoácidos da proteína recombinante com moléculas reconhecidamente alergênicas, levando em conta bancos de dados incompletos (porque em evolução permanente). Também examinam a suposta degradação da proteína transgênica em ambiente gástrico simulado. Análise *in silico* das novas proteínas produzidas pelo OGM e testes de longo prazo com animais modelo não têm sido adotados supostamente por serem considerados excessivamente onerosos para as empresas.

Ademais, a participação potencial do consumo de plantas transgênicas (e agrotóxicos associados) nas explosões dos casos de novas alergias e mesmo em processos de intolerância alimentares, concretizada por ações sinérgicas em reações imunológicas cruzadas, não é analisada pelos tomadores de decisão. Enquanto isso, os casos de novas alergias e intolerâncias assumem proporção de pandemia, paralelamente à expansão das lavouras e dos alimentos geneticamente modificados.

REV - Hodgson, E. 2001. Genetically modified plants and human health risks: Can additional research reduce uncertainties and increase public confidence? *Toxicological Sciences*, 63, 153–156.

So long as the risks to human health from transgenic plants remain potential rather than actual, and, in any event, appear lower than those from traditional plant breeding, hazard assessment need not be extensive. However, in view of current public attitudes to transgenic plants, it is necessary that those tests that are required, be based on logic, on sound science, and in accordance with the best scientific methodology. This is particularly the case with testing for food allergenicity. Current testing is largely indirect and based on comparisons with other known food allergens. Development of direct tests that involve interaction between the actual transgenic protein in question and the immune system is essential if confidence in the regulatory system is to be restored.

Artigo completo disponível em http://toxsci.oxfordjournals.org/content/63/2/153.long

Kleter, G.; Peijnenburg, Ad A. 2002. Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE – binding linear epitopes of allergens. *BMC Structural Biology*, 2:8.

Background: Transgenic proteins expressed by genetically modified food crops are evaluated for their potential allergenic properties prior to marketing, among others by identification of short identical amino acid sequences that occur both in the transgenic protein and allergenic proteins. A strategy is proposed, in which the positive outcomes of the sequence comparison with a minimal length of six amino acids are further screened for the presence of potential linear IgE-epitopes. This double track approach involves the use of literature data on IgE-epitopes and an antigenicity prediction algorithm.
Results: Thirty-three transgenic proteins have been screened for identities of at least six contiguous amino acids shared with allergenic proteins. Twenty-two transgenic proteins showed positive results of six- or seven-contiguous amino acids length. Only a limited number of identical stretches shared by transgenic proteins (papaya ringspot virus coat protein, acetolactate synthase GH50, and glyphosate oxidoreductase) and allergenic proteins could be identified as (part of) potential linear epitopes.
Conclusion: Many transgenic proteins have identical stretches of six or seven amino acids in common with allergenic proteins. Most identical stretches are likely to be false positives. As shown in this study, identical stretches can be further screened for relevance by comparison with linear IgE-binding epitopes described in literature. In the absence of literature data on epitopes, antigenicity prediction by computer aids to select potential antibody binding sites that will need verification of IgE binding by sera binding tests. Finally, the positive outcomes of this approach warrant further clinical testing for potential allergenicity.

Artigo completo disponível em http://www.biomedcentral.com/1472-6807/2/8

Bucchini, L.; Goldman, L. 2002. Starlink corn: A risk analysis. *Environmental Health Perspectives*, Volume 110. Nº 1.

Modern biotechnology has dramatically increased our ability to alter the agronomic traits of plants. Among the novel traits that biotechnology has made available, an important group includes Bacillus thuringiensis-derived insect resistance. This technology has been applied to potatoes, cotton, and corn. Benefits of Bt crops, and biotechnology generally, can be realized only if risks are assessed and managed properly. The case of Starlink corn, a plant modified with a gene that encodes the Bt protein Cry9c, was a severe test of U.S. regulatory agencies. The U.S. Environmental Protection Agency had restricted its use to animal feed due to concern about the potential for allergenicity. However, Starlink corn was later found throughout the human food supply, resulting in food recalls by the Food and Drug Administration and significant disruption of the food supply. Here we examine the regulatory history of Starlink, the assessment framework employed by the U.S. government, assumptions and information gaps, and the key elements of government efforts to manage the product. We explore the impacts on regulations, science, and society and conclude that only significant advances in our understanding of food allergies and improvements in monitoring and enforcement will avoid similar events in the future. Specifically, we need to develop a stronger fundamental basis for predicting allergic sensitization and reactions if novel proteins are to be introduced in this fashion. Mechanisms are needed to assure that worker and community aeroallergen risks are considered. Requirements are needed for the development of valid assays so that enforcement and post market surveillance activities can be conducted.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1240687/pdf/ehp0110-000005.pdf

Sutton, S.; Assa'ad, A.; Steinmetz, C.; Rothenberg, M. 2003. A negative, double-blind, placebo-controlled challenge to genetically modified corn. *J Allergy Clin Immunol*, Volume 112, Number 5.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/14610498

Bernstein, J.; Bernstein, I.; Bucchini, L.; Goldman, L.; Hamilton, R.; Lehrer, S.; Rubin, C.; Sampson, H. 2003. Clinical and laboratory investigation of allergy to genetically modified foods. *Environ Health Perspect*, 111(8): 1114-1121.

Technology has improved the food supply since the first cultivation of crops. Genetic engineering facilitates the transfer of genes among organisms. Generally, only minute amounts of a specific protein need to be expressed to obtain the desired trait. Food allergy affects only individuals with an abnormal immunologic response to food--6% of children and 1.5-2% of adults in the United States. Not all diseases caused by food allergy are mediated by IgE. A number of expert committees have advised the U.S. government and international organizations on risk assessment for allergenicity of food proteins. These committees have created decision trees largely based on assessment of IgE-mediated food allergenicity. Difficulties include the limited availability of allergen-specific IgE antisera from allergic persons as validated source material, the utility of specific IgE assays, limited characterization of food proteins, cross-reactivity between food and other allergens, and modifications of food proteins by processing. StarLink was a corn variety modified to produce a (Italic)Bacillus thuringiensis(/Italic) (Bt) endotoxin, Cry9C. The Centers for Disease Control and Prevention investigated 51 reports of possible adverse reactions to corn that occurred after the announcement that StarLink, allowed for animal feed, was found in the human food supply. Allergic reactions were not confirmed, but tools for postmarket assessment were limited. Workers in agricultural and food preparation facilities have potential inhalation exposure to plant dusts and flours. In 1999, researchers found that migrant health workers can become sensitized to certain Bt spore extracts after exposure to Bt spraying.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1241560/pdf/ehp0111-001114.pdf

2.2 Críticas à falta de rigor científico sobre avaliação do risco toxicológico

A literatura científica acumula documentos críticos às metodologias adotadas na avaliação toxicológica de plantas transgênicas. As fragilidades constatadas permitem que ensaios de curta duração, avaliando, por exemplo, uma proteína recombinante extraída de bactéria (no lugar do alimento inteiro, obtido da PGM), sustentem ausência de problemas que não são observados porque os protocolos o impedem. As críticas avançam demonstrando que os elementos de riscos reais, aos quais os consumidores são submetidos, não estão sendo corretamente avaliados.

Outros artigos salientam que aspectos-chave da toxicologia, tais como a representatividade (qualitativa e quantitativa) dos grupos testes ou a diferenciação dos sexos, nem sempre são contemplados de forma adequada (e por vezes são totalmente desprezados). À busca de uma resposta dose-tratamento – que acaba escondendo desregulações endócrinas ou negligenciando diferenças estatisticamente significativas em variáveis biológicas chaves –, os órgãos reguladores e as empresas de biotecnologia estimulam a obtenção de conclusões favoráveis à tecnologia. Distorções decorrentes dessas e de outras falhas metodológicas acabam sendo utilizadas como argumento para comprovar hipóteses de ausência de riscos para a saúde dos consumidores de PGMs e suas partes.

REV - Pusztai, A. 2002. Can science give us the tools for recognizing possible health risks of GM food? *Nutr Health*, 16, 73-84.

Nearly ten years after the introduction of GM foodcrops there are still only a handful of published studies about their safety. Independent studies are even fewer, moreover, no peer-reviewed publications exist in which the results of clinical investigations on the possible effects of GM food on human health are described. Even though the evaluation of the safety or possible toxicity of GM foodstuffs is more difficult than that of drugs or food additives, this scarcity of data and the lack of a scientific database is curious particularly as descriptions of the results of chemical, nutritional and biological testing in some early (unpublished) studies or some more recent publications demonstrate the feasibility of carrying out proper and scientifically valid health risk assessment on GM foods. In this review, after critically examining some of the basic principles, past results and possible novel methods of future health safety assessment of GM foodstuffs, the conclusion appears to be that as the tools for the recognition and indeed for the elimination of the risks GM foods may present for us are available or can be developed, it is the will and the funding for such work that needs to be found.

http://www.ncbi.nlm.nih.gov/pubmed/12102369

Schrøder, M.; Poulsen, M.; Wilcks, A.; Kroghsbo, S.; Miller, A.; Frenzel, T.; Danier, J.; Rychlik, M.; Emami, K.; Gatehouse, A.; Shu, Q.; Engel, K.; Altosaar, I.; Knudsen, I. 2007. A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. *Food Chem Toxicol*, 45(3): 339-349.

An animal model for safety assessment of genetically modified foods was tested as part of the SAFOTEST project. In a 90-day feeding study on Wistar rats, the transgenic KMD1 rice expressing Cry1Ab protein was compared to its non-transgenic parental wild type, Xiushui 11. The KMD1 rice contained 15mg Bt toxin/kg and based on the average feed consumption the daily intake was 0.54mg Bt toxin/kg body weight. No adverse effects on animal behaviour or weight gain were observed during the study. Blood samples collected one week prior to sacrifice were analyzed and compared for standard haematological and biochemical parameters. A few parameters were significantly different, but all within the normal reference intervals for rats of this breed and age and not in relation to any other findings, thus not considered treatment related. Upon sacrifice a large number of organs were weighed, macroscopic and histopathological examinations were performed with only minor changes to report. The aim of the study was to use a known animal model in performance of safety assessment of a GM crop, in this case KMD1 rice. The results show no adverse or toxic effects of KMD1 rice when tested in the design used in this 90-day study. Nevertheless the experiences from this study lead to the overall conclusion that safety assessment for unintended effects of a GM crop cannot be done without additional test group(s).

http://www.ncbi.nlm.nih.gov/pubmed/17050059

REV - Séralini, G-E.; Vendômois, J.; Cellier, D.; Sultan, C.; Buiatti, M.; Gallagher, L.; Antoniou, M.; Dronamraju, K. 2009. How subchronic and chronic health effects can be neglected for GMOs, pesticides or chemicals. *International Journal of Biological Science*, 5, 438–443.

Chronic health effects are increasing in the world such as cancers, hormonal, reproductive, nervous, or immune diseases, even in young people. During regulatory toxicological subchronic tests to prevent these on mammalian health, prior commercialization of chemicals, including pesticides and drugs, or GMOs, some statistically significant findings may be revealed. This discussion is about the need to investigate the relevant criteria to consider those as biologically significant. The sex differences and the non linear dose or time related effects should be considered in contrast to the claims of a Monsanto-supported expert panel about a GMO, the MON 863 Bt maize, but also for pesticides or drugs, in particular to reveal hormone-dependent diseases and first signs of toxicities.

Artigo completo disponível em http://www.ijbs.com/v05p0438.htm

REV - Vendômois, J.; Roullier, F.; Cellier, D.; Séralini, G-E. 2009. A comparison of the effects of three GM corn varieties on mammalian health. *International Journal of Biological Sciences*, 5: 706-726.

We present for the first time a comparative analysis of blood and organ system data from trials with rats fed three main commercialized genetically modified (GM) maize (NK 603, MON 810, MON 863), which are present in food and feed in the world. NK 603 has been modified to be tolerant to the broad spectrum herbicide Roundup and thus contains residues of this formulation. MON 810 and MON 863 are engineered to synthesize two different Bt toxins used as insecticides. Approximately 60 different biochemical parameters were classified per organ and measured in serum and urine after 5 and 14 weeks of feeding. GM maize-fed rats were compared first to their respective isogenic or parental non-GM equivalent control groups. This was followed by comparison to six reference groups, which had consumed various other non-GM maize varieties. We applied nonparametric methods, including multiple pairwise comparisons with a False Discovery Rate approach. Principal Component Analysis allowed the investigation of scattering of different factors (sex, weeks of feeding, diet, dose and group). Our analysis clearly reveals for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also noticed in the heart, adrenal glands, spleen and haematopoietic system. We conclude that these data highlight signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM corn. In addition, unintended direct or indirect metabolic consequences of the genetic modification cannot be excluded.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2793308/

REV - Vendômois, J.; Cellier, D.; Vélot, C.; Clair, E.; Mesnage, R.; Séralini, G-E. 2010. Debate on GMOs health risks after statistical findings in regulatory tests. *International Journal of Biological* Sciences, 6, 590-598.

We summarize the major points of international debate on health risk studies for the main commercialized edible GMOs. These GMOs are soy, maize and oilseed rape designed to contain new pesticide residues since they have been modified to be herbicide-tolerant (mostly to Roundup) or to produce mutated Bt toxins. The debated alimentary chronic risks may come from unpredictable insertional mutagenesis effects, metabolic effects, or from the new pesticide residues. The most detailed regulatory tests on the GMOs are three-month long feeding trials of laboratory rats, which are biochemically assessed. The tests are not compulsory, and are not independently conducted. The test data and the corresponding results are kept in secret by the companies. Our previous analyses of regulatory raw data at these levels, taking the representative examples of three GM maize NK 603, MON 810, and MON 863 led us to conclude that hepatorenal toxicities were possible, and that longer testing was necessary. Our study was criticized by the company developing the GMOs in question and the regulatory bodies, mainly on the divergent biological interpretations of statistically significant biochemical and physiological effects. We present the scientific reasons for the crucially different biological interpretations and also highlight the shortcomings in the experimental protocols designed by the company. The debate implies an enormous responsibility towards public health and is essential due to nonexistent traceability or epidemiological studies in the GMO-producing countries.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952409/

REV – Séralini, G-E.; Mesnage, R.; Clair, E.; Gress, S.; Vendômois, J.; Cellier, D. 2011. Genetically modified crops safety assessments: present limits and possible improvements. *Environmental Sciences Europe*, 23:10.

Purpose: We reviewed 19 studies of mammals fed with commercialized genetically modified soybean and maize which represent, per trait and plant, more than 80% of all environmental genetically modified organisms (GMOs) cultivated on a large scale, after they were modified to tolerate or produce a pesticide. We have also obtained the raw data of 90-day-long rat tests following court actions or official requests. The data obtained include biochemical blood and urine parameters of mammals eating GMOs with numerous organ weights and histopathology findings.
Methods: We have thoroughly reviewed these tests from a statistical and a biological point of view. Some of these tests used controversial protocols which are discussed and statistically significant results that were considered as not being biologically meaningful by regulatory authorities, thus raising the question of their interpretations.
Results: Several convergent data appear to indicate liver and kidney problems as end points of GMO diet effects in the above-mentioned experiments. This was confirmed by our meta-analysis of all the in vivo studies published, which revealed that the kidneys were particularly affected, concentrating

43.5% of all disrupted parameters in males, whereas the liver was more specifically disrupted in females (30.8% of all disrupted parameters).
Conclusions: The 90-day-long tests are insufficient to evaluate chronic toxicity, and the signs highlighted in the kidneys and livers could be the onset of chronic diseases. However, no minimal length for the tests is yet obligatory for any of the GMOs cultivated on a large scale, and this is socially unacceptable in terms of consumer health protection. We are suggesting that the studies should be improved and prolonged, as well as being made compulsory, and that the sexual hormones should be assessed too, and moreover, reproductive and multigenerational studies ought to be conducted too.

Artigo completo disponível em http://www.enveurope.com/content/23/1/10

Snell, C.; Bernheim, A.; Bergé, J.; Kuntz, M.; Pascal, G.; Paris, A.; Ricroch, A. 2012. Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. *Food and Chemical Toxicology*, 50(3-4):1134-48. doi: 10.1016/j.fct.2011.11.048.

The aim of this systematic review was to collect data concerning the effects of diets containing GM maize, potato, soybean, rice, or triticale on animal health. We examined 12 long-term studies (of more than 90 days, up to 2 years in duration) and 12 multigenerational studies (from 2 to 5 generations). We referenced the 90-day studies on GM feed for which long-term or multigenerational study data were available. Many parameters have been examined using biochemical analyses, histological examination of specific organs, hematology and the detection of transgenic DNA. The statistical findings and methods have been considered from each study. Results from all the 24 studies do not suggest any health hazards and, in general, there were no statistically significant differences within parameters observed. However, some small differences were observed, though these fell within the normal variation range of the considered parameter and thus had no biological or toxicological significance. If required, a 90-day feeding study performed in rodents, according to the OECD Test Guideline, is generally considered sufficient in order to evaluate the health effects of GM feed. The studies reviewed present evidence to show that GM plants are nutritionally equivalent to their non-GM counterparts and can be safely used in food and feed.

http://www.ncbi.nlm.nih.gov/pubmed/22155268

REV – Heinemann, J.; Agapito-Tenfen, S.; Carman, J. 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. *Environment International*, Vol. 55: 43-55.

Changing the nature, kind and quantity of particular regulatory-RNA molecules through genetic engineering can create biosafety risks. While some genetically modified organisms (GMOs) are intended to produce new regulatory-RNA molecules, these may also arise in other GMOs not intended to express them. To characterise, assess and then mitigate the potential adverse effects arising from changes to RNA requires changing current approaches to food or environmental risk assessments of GMOs. We document risk assessment advice offered to government regulators in Australia, New Zealand and Brazil during official risk evaluations of GM plants for use as human food or for release into the environment (whether for field trials or commercial release), how the regulator considered those risks, and what that experience teaches us about the GMO risk assessment framework. We also suggest improvements to the process.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0160412013000494

Meyer, H.; Hilbeck, A. 2013. Rat feeding studies with genetically modified maize – a comparative evaluation of applied methods and risk assessment standards. *Environmental Sciences Europe*, 25:33 (doi:10.1186/2190-4715-25-33).

A 2-year rat feeding study with genetically modified NK603 maize sparked an international scientific and public debate as well as policy responses by the European Commission. The European Food Safety Authority (EFSA) evaluated the study as defective based on conceptual and methodological shortcomings by retroactive application of the recommendations of its recent guidance on 90-day feeding studies. Our comparative analysis of the three relevant NK603 publications, including a 90-day feeding study of Monsanto, showed that all of them satisfy or fail to satisfy the EFSA evaluation criteria to a comparable extent; the rejection of only one of the papers is, thus, not scientifically justified. We also show that EFSA's criteria are not standard practice in 21 other rat feeding studies lasting at a minimum of 12 months. The review reveals critical double standards in the evaluation of feeding studies submitted as proof of safety for regulatory approval to EFSA. We specifically argue that the current approach to declare statistically significant differences between genetically modified organisms and its parents as 'biologically irrelevant' based on additional reference controls lacks scientific rigor and legal justification in the European Union (EU) system. Only recently, the EU authorities started building up an implementing system based on its own legislation and supportive of the EU approach to risk assessment in the context of technology assessment. Until these issues are resolved, we do not expect that neither the public nor the scientific debate will subside.

Artigo completo disponível em http://www.enveurope.com/content/25/1/33

COM – Mesnage, R.; Defarge, N.; Vendômois, J.; Séralini, G-E. 2014. Letter to the editor regarding "Delaney et al., 2014": uncontrolled GMOs and their associated pesticides make the conclusions unreliable. *Food Chem Toxicol*, 72:322. doi: 10.1016/j.fct.2014.07.003.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/24997312

É relevante o fato de que a polêmica científica em relação à avaliação de riscos toxicológicos dos agrotóxicos, para a saúde humana e o meio ambiente, assume caráter de quase consenso. Não há mais dúvidas a respeito de um fato básico: a aplicação de venenos sobre plantas destinadas ao consumo trará implicações negativas para a saúde das populações e dos organismos a que se destinam.

Ainda assim, para parte significativa (mas não para a totalidade) da comunidade científica e para formadores de opinião com espaço na grande mídia, os ensaios e as avaliações simplificados, tal como conduzidos pelos órgãos reguladores, são suficientes. Para outro grupo de pesquisadores, assim como para a população em geral, os métodos analíticos atendem a interesses econômicos e ameaçam a credibilidade das instituições.

Estudos disponíveis revelam a presença de metodologias inadequadas, incapazes de representar riscos efetivamente associados aos agrotóxicos e seus resíduos em alimentos de modo geral. Conceitos de uso generalizado, como o LMR (Limite Máximo de Resíduo aceitável) e o IDA (Ingestão Diária Aceitável), não levam em conta sinergias, adjuvantes, combinações e superposição de alimentos elaborados com OGMs ou ainda diversidade biológica dos consumidores (idade, doenças, hipersensibilidade, etc.).

REV - Colborn, T. 2006. A case for revisiting the safety of pesticides: a closer look at neurodevelopment. *Environmental Health Perspectives*, 114(1): doi:10.1289/ehp.7940.

The quality and quantity of the data about the risk posed to humans by individual pesticides vary considerably. Unlike obvious birth defects, most developmental effects cannot be seen at birth or even later in life. Instead, brain and nervous system disturbances are expressed in terms of how an individual behaves and functions, which can vary considerably from birth through adulthood. In this article I challenge the protective value of current pesticide risk assessment strategies in light of the vast numbers of pesticides on the market and the vast number of possible target tissues and end points that often differ depending upon timing of exposure. Using the insecticide chlorpyrifos as a model, I reinforce the need for a new approach to determine the safety of all pesticide classes. Because of the uncertainty that will continue to exist about the safety of pesticides, it is apparent that a new regulatory approach to protect human health is needed.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1332649/

Hardell, L. 2008. Pesticides, soft-tissue sarcoma and non-Hodgkin lymphoma - historical aspects on the precautionary principle in cancer prevention. *Acta Oncologica*, 47: 347-354.

Background. After the 2nd World War a long range of chemical agents have been introduced on the market, both in Sweden and most other countries. From the 1950's several pesticides gained increasing use in agriculture and forestry. In the 1970's public concern increased in Sweden especially regarding use of phenoxy herbicides to combat deciduous wood, although statements from different authorities were reassuring of the safety. *Materials and methods.* At the end of the 1970's the author and his colleagues published the first scientific evidence of an association between exposure to phenoxyacetic acids, chlorophenols and certain malignant tumours, i.e., soft-tissue sarcoma and malignant lymphoma. The study subjects were also exposed to contaminating dioxins such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Later studies showed also an association between certain persistent organic pollutants such as polychlorinated biphenyls and non-Hodgkin lymphoma (NHL) with an interaction with titers of antibodies to Epstein-Barr virus early antigen. These results have been corroborated in other studies. *Discussion.* Over the years industry and its allied experts have attacked our studies, but in 1997 IARC classified TCDD as a human carcinogen, Group I. The increasing incidence of NHL in Sweden levelled off about 1990. The author postulated that the regulation or ban of the use of chlorophenols, certain phenoxy herbicides and some persistent organic pollutants in Sweden back in the 1970s has contributed to the now decreasing incidence of NHL. Unfounded criticism from industry experts may prohibit the precautionary principle and early warnings of cancer risk can be ignored. Cancer risks by certain chlorinated phenols may serve as a model of how the precautionary principle should be used by taking early warnings seriously.

Artigo completo disponível em http://informahealthcare.com/doi/pdf/10.1080/02841860701753697

Tennekes, H. 2010. The significance of the Druckrey-Küpfm**ü**ller equation for risk assessment--the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. *Toxicology*, 276(1): 1-4.

The essence of the Druckrey-Küpfmüller equation dtn = constant (where d = daily dose and t = exposure time-to-effect, with n > 1) for chemical carcinogens is that the total dose required to produce the same effect decreases with decreasing exposure levels, even though the exposure times required to produce the same effect increase with decreasing exposure levels. Druckrey and Küpfmüller inferred that if both receptor binding and the effect are irreversible, exposure time would reinforce the effect. The Druckrey-Küpfmüller equation explains why toxicity may occur after prolonged exposure to very low toxicant levels. Recently, similar dose-response characteristics have been established for the toxicity of the neonicotinoid insecticides imidacloprid and thiacloprid to arthropods. This observation is highly relevant for environmental risk assessment. Traditional approaches that consider toxic effects at fixed exposure times are unable to allow extrapolation from measured endpoints to effects that may occur at other times of exposure. Time-to-effect approaches that provide information on the doses and exposure times needed to produce toxic effects on tested organisms are required for prediction of toxic effects for any combination of concentration and time in the environment.

http://www.ncbi.nlm.nih.gov/pubmed/20803795

Mesnage, R.; Defarge, N.; Vendômois, J.; Séralini, G-E. 2014. Major pesticides are more toxic to human cells than their declared active principles. *BioMed Research International*, vol. 2014, Article ID 179691, 8 pages. doi:10.1155/2014/179691.

Pesticides are used throughout the world as mixtures called formulations. They contain adjuvants, which are often kept confidential and are called inerts by the manufacturing companies, plus a declared active principle (AP), which is usually tested alone. This is true even in the longest toxicological regulatory tests performed on mammals. We tested the toxicity of 9 pesticides, comparing active principles and their formulations, on three human cell lines (HepG2, HEK293 and JEG3). We measured mitochondrial activities, membrane degradations, and caspases 3/7 activities. Glyphosate, isoproturon, fluroxypyr, pirimicarb, imidacloprid, acetamiprid, tebuconazole, epoxiconazole and prochloraz constitute respectively the active principles of 3 major herbicides, 3 insecticides and 3 fungicides. Fungicides were the most toxic from concentrations 300-600 times lower than agricultural dilutions, followed by herbicides, and then insecticides, with very similar profiles in all cell types. The human placental JEG3 cells appeared to be the most sensitive. Despite its relatively benign reputation, Roundup was by far the most toxic among the herbicides and insecticides tested. Most importantly, 8 formulations out of 9 were several hundred times more toxic than their active principle. Our results challenge the relevance of the Acceptable Daily Intake for pesticides because this norm is calculated from the toxicity of the active principle alone. The study of combinatorial effects of several APs together may be of only secondary importance if the toxicity of the combinations of each AP with its adjuvants is neglected or unknown. Chronic tests on pesticides may not reflect relevant environmental exposures if only one ingredient of these mixtures is tested alone.

Artigo completo disponível em http://www.hindawi.com/journals/bmri/2014/179691/

3 Falta de rigor científico na avaliação do risco para o meio ambiente (ONAs em especial)

A avaliação de riscos ambientais para plantas transgênicas – tal como realizada pelos órgãos reguladores – também tem recebido fortes críticas por parte significativa da comunidade científica. Estudos mostram erros básicos na escolha de organismos não alvo, na cobertura de agroecossistemas, na representatividade das tecnologias tradicionais e na seleção de formas de manejo para comparações de impacto. Ambientes aquáticos são geralmente desprezados, comunidades da microbiota são frequentemente ignoradas e até insetos inexistentes nos biomas onde serão cultivadas as PGMs são utilizados como referências locais – pelo simples motivo de serem de fácil criação em laboratório. Cabe destacar que o conhecimento científico sobre protocolos específicos de avaliação do risco ambiental em meio tropical – em especial na América do Sul – é quase nulo.

Em paralelo, as redes tróficas, as funções ecológicas dos organismos afetados, os balanços populacionais e mesmo as flutuações climáticas naturais são subdimensionados, em avaliações expeditas, de curto prazo e escassa utilidade no que respeita à avaliação do risco. Enfim, as próprias metodologias empregadas em bioensaios para avaliação do risco subcrônico em ONA são contestáveis, como ressaltado nos artigos a seguir.

REV - O'Callaghan, M.; Glare, T.; Burgess, E.; Malone, L. 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. *Annual Review of Entomology*, 50, pp. 271-292.

Insect resistance, based on Bacillus thuringiensis (Bt) endotoxins, is the second most widely used trait (after herbicide resistance) in commercial genetically modified (GM) crops. Other modifications for insect resistance, such as proteinase inhibitors and lectins, are also being used in many experimental crops. The extensive testing on nontarget plant-feeding insects and beneficial species that has accompanied the long-term and wide-scale use of Bt plants has not detected significant adverse effects. GM plants expressing other insect-resistant proteins that have a broader spectrum of activity have been tested on only a limited number of nontarget species. Little is known about the persistence of transgene-derived proteins in soil, with the exception of Bt endotoxins, which can persist in soil for several months. Bt plants appear to have little impact on soil biota such as earthworms, collembolans, and general soil microflora. Further research is required on the effects of GM plants on soil processes such as decomposition. Assessment of nontarget impacts is an essential part of the risk assessment process for insect-resistant GM plants.

http://www.ncbi.nlm.nih.gov/pubmed/15355241

MOD - Andow, D.; Zwahlen, C. 2006. Assessing environmental risks of transgenic plants. *Ecology Letters*, (2): 196-214.

By the end of the 1980s, a broad consensus had developed that there were potential environmental risks of transgenic plants requiring assessment and that this assessment must be done on a case-by-case basis, taking into account the transgene, recipient organism, intended environment of release, and the frequency and scale of the intended introduction. Since 1990, there have been gradual but substantial changes in the environmental risk assessment process. In this review, we focus on changes in the assessment of risks associated with non-target species and biodiversity, gene flow, and the evolution of resistance. Non-target risk assessment now focuses on risks of transgenic plants to the intended local environment of release. Measurements of gene flow indicate that it occurs at higher rates than believed in the early 1990s, mathematical theory is beginning to clarify expectations of risks associated with gene flow, and management methods are being developed to reduce gene flow and possibly mitigate its effects. Insect pest resistance risks are now managed using a high-dose/refuge or a refuge-only strategy, and the present research focuses on monitoring for resistance and encouraging compliance to requirements. We synthesize previous models for tiering risk assessment and propose a general model for tiering. Future transgenic crops are likely to pose greater challenges for risk assessment, and meeting these challenges will be crucial in developing a scientifically coherent risk assessment framework. Scientific understanding of the factors affecting environmental risk is still nascent, and environmental scientists need to help improve environmental risk assessment.

http://www.ncbi.nlm.nih.gov/pubmed/16958885

Perry, J.; Braak, C.; Dixon, P.; Duan, J.; Hails, R.; Huesken, A.; Lavielle, M.; Marvier, M.; Scardi, M.; Schmidt, K.; Tothmeresz, B.; Schaarschmidt, F.; van der Voet, H. 2009. Statistical aspects of environmental risk assessments of GM plants for effects on non-target organisms. *Environ Biosafety*, Res 8: 65–78.

Previous European guidance for environmental risk assessment of genetically modified plants emphasized the concepts of statistical power but provided no explicit requirements for the provision of statistical power analyses. Similarly, whilst the need for good experimental designs was stressed, no minimum guidelines were set for replication or sample sizes. Furthermore, although substantial equivalence was stressed as central to risk assessment, no means of quantification of this concept was given. This paper suggests several ways in which existing guidance might be revised to address these problems. One approach explored is the ;bioequivalence' test, which has the advantage that the error of most concern to the consumer may be set relatively easily. Also, since the burden of proof is placed on the experimenter, the test promotes high-quality, well-replicated experiments with sufficient statistical power. Other recommendations cover the specification of effect sizes, the choice of appropriate comparators, the use of positive controls, meta-analyses, multivariate analysis and diversity indices. Specific guidance is suggested for experimental designs of field trials and their statistical analyses. A checklist for experimental design is proposed to accompany all environmental risk assessments.

Artigo completo disponível em http://journals.cambridge.org/download.php?file=%2FEBS%2FEBS8_02%2FS1635792209000098a.pdf&code=1f707e28da8371773d961c531b3262e2

Liu, W. 2009. Effects of Bt transgenic crops on soil ecosystems: a review of a ten-year research in China. *Frontiers of Agriculture in China*, 3 (2): 190–198.

Bacillus thuringiensis (Bt) transgenic cotton is the unique Bt transgenic crop planted on a large scale in China, and its commercialized varieties and hectareage had increased rapidly in China during the past decade (1997–2006) with broad geographic distribution for the economic, environmental, and health benefits. In 2004, the planting area of Bt transgenic cotton in China ranked first worldwide with up to 370 × 106 hm2. In addition, Bt transgenic rice varieties in field tests have been close to approval for commercialization. However, ecological risks, a complex issue of Bt transgenic crops on soil ecosystem is urgently faced in China due to more than 60 varieties transferred single or bivalent Bt genes grown under diverse geographic regions. Two main pathways, biomass incorporation and root exudates, are involved in the effects of Bt transgenic crops on soil ecosystems. In this paper, the research results in recent years in China involved in the effects of Bt transgenic crops (Bt transgenic cottons and rice) on soil ecosystems were summarized with special attentions paid to the release and persistence of Bt toxins, and the toxicology to microorganisms, as well as the change of soil biochemical properties in soils where Bt transgenic crops were planted or incubated with their biomass. In addition, the complexity and current research defaults of ecological risk evaluation of Bt transgenic crops in China were highlighted.

http://link.springer.com/article/10.1007%2Fs11703-009-0027-9

REV - Lang, A.; Otto, M. 2010. A synthesis of laboratory and field studies on the effects of transgenic *Bacillus thuringiensis* (Bt) maize on non-target *Lepidoptera*. *Entomologia Experimentalis et Applicata*, 135 (2): 121134.

One of the major applications of transgenic crops in agriculture are the so-called Bacillus thuringiensis Berliner (Bt) plants, in particular Bt maizes, which produce insecticidal Cry proteins that target specific orders, such as the Lepidoptera or Coleoptera. We reviewed publications that reported on the direct toxic effects of Bt-maize and/or Cry proteins of current Bt-maize events on larvae of non-target butterflies and moths (Lepidoptera). In total, 20 peer-reviewed publications were identified, of which 16 papers contributed laboratory-based data and seven field-based data. An adverse effect on caterpillars was recorded in 52% of all laboratory-based and in 21% of all field-based observations. The variables most often studied and having the highest occurrence of effects were larval survival, body mass, and developmental time. Parameters of the adult stage were under-represented in the studies. Overall, 11 lepidopteran species were tested. The majority of the studies originated from the USA, with the Monarch butterfly being the most studied, whereas other species and other parts of the world were widely neglected. Laboratory experiments were often run under unrealistic conditions from an ecological point of view. Although the papers we reviewed indicated a potential hazard for Lepidoptera that are exposed to and feed on lepidopteran-specific Bt-maize pollen, a general conclusion on the level of risk for butterflies and moths cannot as yet be drawn. A comprehensive risk characterization would require thorough hazard identification, exposure assessment, and impact assessment. However, our review showed that even the basic level of hazard characterization is as yet incomplete. Reasons for this are the still-limited numbers of publications and concurrent lack of knowledge, the restriction of data to only a few species, the over-representation of North American species, and the identified limitations of both laboratory and field experiments. The findings of this review suggest that more realistic, ecologically meaningful, and detailed experiments and analyses are crucial to improve the present assessment of Bt-maize cultivation effects on Lepidoptera.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/j.1570-7458.2010.00981.x/pdf

COM - Lang, A., S. Brunzel, M. Dolek, M. Otto and B. Theißen, 2011. Modelling in the light of uncertainty of key parameters: a call to exercise caution in field predictions of Bt-maize effects. *Proceedings of the Royal Society B*.

Sem resumo.

Artigo completo disponível em http://rspb.royalsocietypublishing.org/content/early/2011/01/04/rspb.2010.2085.full.pdf+html

Hilbeck, A.; Meier, M.; Römbke, J.; Jänsch, S.; Teichmann, H.; Tappeser, B. 2011. Environmental risk assessment of genetically modified plants - concepts and controversies. *Environmental Sciences Europe*, 23(13).

Background and purpose: In Europe, the EU Directive 2001/18/EC lays out the main provisions of environmental risk assessment (ERA) of genetically modified (GM) organisms that are interpreted very differently by different stakeholders. The purpose of this paper is to: (a) describe the current implementation of ERA of GM plants in the EU and its scientific shortcomings, (b) present an improved ERA concept through the integration of a previously developed selection procedure for identification of non-target testing organisms into the ERA framework as laid out in the EU Directive 2001/18/EC and its supplement material (Commission Decision 2002/623/EC), (c) describe the activities to be carried out in each component of the ERA and (d) propose a hierarchical testing scheme. Lastly, we illustrate the outcomes for three different crop case examples.
Main features: Implementation of the current ERA concept of GM crops in the EU is based on an interpretation of the EU regulations that focuses almost exclusively on the isolated bacteria-produced novel proteins with little consideration of the whole plant. Therefore, testing procedures for the effect assessment of GM plants on non-target organisms largely follow the ecotoxicological testing strategy developed for pesticides. This presumes that any potential adverse effect of the whole GM plant and the plant-produced novel compound can be extrapolated from testing of the isolated bacteria-produced novel compound or can be detected in agronomic field trials. This has led to persisting scientific criticism.
Results: Based on the EU ERA framework, we present an improved ERA concept that is system oriented with the GM plant at the centre and integrates a procedure for selection of testing organisms that do occur in the receiving environment. We also propose a hierarchical testing scheme from laboratory studies to field trials and we illustrate the outcomes for three different crop case examples.
Conclusions and recommendations: Our proposed concept can alleviate a number of deficits identified in the current approach to ERA of GM plants. It allows the ERA to be tailored to the GM plant case and the receiving environment.

Artigo completo disponível em http://www.enveurope.com/content/23/1/13

Meyer, H. 2011. Systemic risks of genetically modified crops: the need for new approaches to risk assessment. *Environmental Sciences Europe*, 23:7.

Purpose: Since more than 25 years, public dialogues, expert consultations and scientific publications have concluded that a comprehensive assessment of the implications of genetic engineering in agriculture and food production needs to include health, environmental, social and economical aspects, but only very few legal frameworks allow to assess the two latter aspects. This article aims to explain the divergence between societal debate and biosafety legislation and presents approaches to bring both together.
Main features: The article reviews the development of biosafety regulations in the USA and the EU, focussing on diverging concepts applied for assessing the risks of genetically modified organisms (GMOs).
Results: The dominant environmental risk assessment methodology has been developed to answer basic questions to enable expedient decision making. As a first step, methodologies that take into account complex environmental and landscape aspects should be applied. Expanding the scope of risk assessment, more holistic concepts have been developed, for example the Organisation for Econonomic Co-operation and Development (OECD) concept of systemic risks which includes

socio-economic aspects. International bodies as the OECD, the Convention on Biological Diversity (CBD) and the European Union (EU) have developed the Strategic Environmental Assessment (SEA) as an instrument that includes the additional aspects of risk assessment as demanded by many stakeholders. Interestingly, there had been no attempts yet to link the existing frameworks of GMO risk assessment and SEA.
Conclusions: It is recommended to adapt current models of SEA to assess the systemic risks of GMOs. It is also suggested to revise the EU GMO legislation to promote the inclusion of SEA elements.

Artigo completo disponível em http://www.enveurope.com/content/23/1/7

COM – Hilbeck, A.; Meier, M.; Trtikova, M. 2012. Underlying reasons of the controversy over adverse effects of Bt toxins on lady beetle and lacewing larvae. *Environmental Sciences Europe*, 24:9.

We outline important underlying reasons that fuel the decades-long controversy over adverse effects of Bt toxins expressed in genetically modified plants on beneficial, nontarget organisms. Inconsistent evaluation standards and asymmetrical levels of scrutiny applied to studies reporting significant adverse effects compared to those finding no adverse effects are described using the examples of the green lacewing (Chrysoperla carnea) and the two-spotted lady beetle (Adalia bipunctata). Additionally, the chosen style and concerted nature of the rather confrontational counter study and responses in the lady beetle cases bear striking similarities to other reported examples in the field of biosafety/risk science of genetically modified plants and to other fields of applied industrial techno-science that suggest deeper issues that go well beyond science. We call for a constructive and respectful scientific discourse where moving the frontiers of our collective knowledge forward takes center stage. Reported phenomena based on robust data must not be rejected or delegitimized on their being surprising and lacking an explained mechanism at the time of their discovery. Exploring mechanisms often requires entirely different expertise and methodologies than those of the discoverers. In particular, in biosafety/risk sciences, plurality of arguments and critical research approaches have to be embraced and actively encouraged rather than discredited or even silenced if we are to learn our 'late lessons' from past technology introductions.

Artigo completo disponível em http://www.enveurope.com/content/24/1/9

Heinemann, J.; El-Kawy, O. 2012. Observational science in the environmental risk assessment and management of GMOs. *Environment International*, 45 (2012) 68–71.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/22575805

COM - Wickson, F.; Bøhn, T.; Wynne, B.; Hilbeck, A.; Funtowicz, S. 2013. Science-based risk assessment requires careful evaluation of all studies. *Nature Biotechnology*, Volume 31, Number 12, 1077-1078.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/24316639

Gillund, F.; Nordgaard, L.; Bøhn, T.; Wikmark, O.; Konestabo, H.; Hilbeck, A. 2014. Selection of nontarget testing organisms for ERA of GM potato with increased resistance to late blight. *Eur J Potato Research*, 56, (4), pp 293-324 DOI: 10.1007/s11540-013-9245-x.

The deliberate release of any genetically modified (GM) organism in the European Union requires an environmental risk assessment (ERA) prior to commercialisation, including impact assessment on nontarget organisms. We report from two expert workshops where a newly developed selection procedure for identification of ecologically relevant testing organisms was applied to the case of a GM potato with increased resistance to late blight, planned for cultivation in southern Scandinavia. Species known to contribute to important ecological functions in the receiving environment were selected in a stepwise procedure, to arrive at a practical number of ecologically relevant species that are likely to be exposed to the transgene and suitable for experimental testing. Four ecological functional categories were identified: herbivory and disease transmission, natural enemies, ecological soil processes and pollination. Among these, relevant nontarget species were identified for herbivores and soil living pathogens, natural enemies and decomposers/beneficial soil organisms. Out of a total of 16 herbivores, 17 soil-living pathogens, 49 natural enemies and 14 decomposers/beneficial soil organisms in the initial lists, 8 herbivores, 10 soil-living pathogens, 15 natural enemies and 11 decomposers/beneficial soil organisms were identified as possible testing organisms, based on ecological criteria. These findings are highly relevant for determining the scope and structure of an ERA of this type of GM potato. The selection procedure could not be completed because of insufficient information about tissue- and developmental stage-specific expression levels of the transgenic products for this particular GM potato. Thus, the case study illustrates some of the difficulties and knowledge gaps that limit the relevance and quality of ERA of GM plants.

http://link.springer.com/article/10.1007%2Fs11540-013-9245-x

4 Polêmicas científicas e campanhas que visam a “atirar no mensageiro”

Nos anos recentes, a literatura científica incorpora procedimento raro, em períodos que antecederam a emergência das biotecnologias. Em paralelo às críticas sobre metodologias “oficiais” de análise de risco, acumulam-se polêmicas orientadas a atingir – se possível desmoralizar – estudiosos e centros de pesquisa que discordam da visão dominante produzida pelas empresas e/ou associadas a seus interesses.

Essas polêmicas são sistematicamente desencadeadas em direção a artigos que observam ou sugerem impactos negativos de plantas transgênicas, independentemente do domínio da biologia considerado. O item a seguir incorpora farta documentação a respeito desses mecanismos. Seus resultados contribuem para o obscurantismo científico, ampliando a margem de riscos que ameaça a saúde do ambiente e dos consumidores de PGMs.

4.1 Campanhas de descredibilização relatadas na literatura científica e controle da pesquisa pelas empresas de biotecnologias

COM - *The Lancet Editorial*, 1999. Health risks of genetically modified foods. Volume 353, No. 9167, p1811, 29 May 1999.

Sem resumo.

Artigo completo disponível em http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(99)00093-8/fulltext

COM - Ewen, S.; Pusztai, A. 1999. Health risks of genetically modified foods. *The Lancet*, Volume 354, No. 9179, p684, 21.

Sem resumo.

Artigo completo disponível em http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(05)77668-6/fulltext

Waltz, E. 2009. Under wraps. *Nature Biotechnology*, 27(10): 880-882.

Are the crop industry's strong-arm tactics and close-fisted attitude to sharing seeds holding back independent research and undermining public acceptance of transgenic crops?

http://www.nature.com/nbt/journal/v27/n10/abs/nbt1009-880.html

Waltz, E. 2009. GM crops: Battlefield. *Nature*, 461: 27-32.

Papers suggesting that biotech crops might harm the environment attract a hail of abuse from other scientists. Emily Waltz asks if the critics fight fair.

Artigo completo disponível em http://www.nature.com/news/2009/090902/full/461027a.html

Lotter, D. 2007. The genetic engineering of food and the failure of science – Part 1: The development of a flawed enterprise. *Int Jrnl of Soc of Agr & Food*, 16(1): 31–49.

A major conflict has developed in science and society between promoters and opponents of transgenic foods. Food, feed, and fiber products derived from transgenic agricultural crops are presented here as a different case from bacterial, industrial, and pharmaceutical crop transgenics and should be parsed from the larger transgenics industry for comprehensive re-evaluation and market roll-back. Reviewed is the development of the crop transgenics industry; the early influence of the biotechnology industry over the US federal regulatory agencies in the context of the development of minimal regulation; the basic technology of plant transgenics; the main transgenic crops, traits, and producing countries; consumer resistance to transgenic foods; industry problems with shrinking investments; the worldwide promotion of transgenic crops; and ecological issues of transgenic crops. Flaws in the one gene–one protein model, the foundation of transgenics, are reviewed in the context of the recent and ongoing restructuring of the science of genetics. Research on the mutational consequences of plant transgenics and its phenotypic ramifications such as allergens and novel proteins is discussed. Major research findings and 'red flag' incidents in the history of transgenic foods and feeds are reviewed that reflect the flaws in the genetic foundations of transgenics.

Artigo completo disponivel em http://ijsaf.org/archive/16/1/lotter1.pdf

Lotter, D. 2008. The genetic engineering of food and the failure of science – Part 2: Academic capitalism and the loss of scientific integrity. *Int Jrnl of Soc of Agr & Food*, 16(1): 50–68.

Factors in the failure of the scientific community to properly oversee agricultural transgenics are presented. The large-scale restructuring of university science programs in the past 25 years from a model based on non-proprietary science for the 'public good' to the 'academic capitalism' model based on the 'knowledge economy' is discussed in the context of the failure of the science community to oversee the transition of transgenic crop technology from the research stage to commercialization. Discussed are increasing science community and university dependence on private industry funding and on development of proprietary technologies; monopolization of the make-up of expert scientific bodies on transgenics by pro-industry scientists with vested interests in transgenics; deficient scientific protocols, bias, and possible fraud in industry-sponsored and industry-conducted research; increasing politically and commercially driven manipulation of science within federal regulatory bodies such as the FDA; and bias in the peer-review process, tolerance by the scientific community of biotechnology industry manipulation of the information environment, and of biased treatment and harassment of non-compliant scientists. Discussed are future food production strategies for developing countries, recently framed in the 2008 UNsponsored International Assessment of Agricultural Knowledge, Science, and Technology, an action plan that emphasizes non-proprietary, agroecology-based approaches to food production and does not include crop transgenics as a central strategy. The under-funding of non-proprietary agroecological approaches to food production is discussed.

Artigo completo disponível em http://www.ijsaf.org/archive/16/1/lotter2.pdf

4.2 Exemplos de campanhas que visam a "atirar no mensageiro"

Várias vezes, na história da pesquisa sobre impactos das plantas transgênicas sobre o meio ambiente e a saúde humana e animal, artigos que apontavam para riscos de OGM foram imediatamente e severamente criticados por determinados componentes da comunidade científica. Por vezes, em consequência de tais campanhas, as críticas e os achados originais resultam amortecidos, sendo necessários vários anos e lento acúmulo de novas evidências, reiteradas em artigos confirmadores, para que argumentos antecipados pelos primeiros autores venham a ser aceitos e reconhecidos como de fundamentação científica. Nesses casos, os detratores não são lembrados, e os prejuízos decorrentes do desprezo a informações relevantes para a saúde humana e ambiental não são atribuídos a quem quer que seja.

4.2.1 Efeitos negativos sobre ONAs: o caso das borboletas Monarca

Em 1999, no início da comercialização em grande escala das plantas Bt, Losey e colaboradores anteciparam problemas para as populações da borboleta Monarca (espécie migratória e de valor simbólico nos EUA). Ao desenvolver parte do seu ciclo de vida em proximidade de lavouras Bt, aquelas populações estariam sob risco. Rapidamente os autores sofreram campanha de difamação. Ainda assim, após várias publicações sobre o tema, impactos negativos foram confirmados. Atualmente a borboleta Monarca é usada como espécie modelo para testes de impactos ambientais daquelas toxinas, sobre ONAs.

COM – Losey, J.; Rayor, L.; Carter, M. 1999. Transgenic pollen harms monarch larvae. *Nature*, 399 (6733): 214.

Sem resumo.

Artigo completo disponível em http://www.nature.com/scitable/content/transgenic-pollen-harms-monarch-larvae-97961

Jesse, L.; Obrycki, J. 2000. Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia*, 125:241–248. doi: 10.1007/s004420000502.

We present the first evidence that transgenic Bacillus thuringiensis (Bt) corn pollen naturally deposited on Asclepias syriaca; common milkweed, in a corn field causes significant mortality of Danaus plexippus L. (Lepidoptera: Danaidae) larvae. Larvae feeding for 48 h on A. syriaca plants naturally dusted with pollen from Bt corn plants suffered significantly higher rates of mortality at 48 h (20±3%) compared to larvae feeding on leaves with no pollen (3±3%), or feeding on leaves with non-Bt pollen (0%). Mortality at 120 h of D. plexippus larvae exposed to 135 pollen grains/cm(2) of transgenic pollen for 48 h ranged from 37 to 70%. We found no sub-lethal effects on D. plexippus adults reared from larvae that survived a 48-h exposure to three concentrations of Bt pollen. Based on our quantification of the wind dispersal of this pollen beyond the edges of agricultural fields, we predict that the effects of transgenic pollen on D. plexippus may be observed at least 10 m from transgenic field borders. However, the highest larval mortality will likely occur on A. syriaca plants in corn fields or within 3 m of the edge of a transgenic corn field. We conclude that the ecological effects of transgenic insecticidal crops need to be evaluated more fully before they are planted over extensive areas.

http://www.ncbi.nlm.nih.gov/pubmed/24595835

Pleasants, J.; Hellmich, R.; Dively, G.; Sears, M.; Stanley-Horn, D.; Mattila, H.; Foster, J.; Clark, P.; Jones, G. 2001. Corn pollen deposition on milkweeds in and near cornfields. *PNAS*, vol. 98, no. 21, 11919–11924.

The density of corn pollen on leaves of milkweed plants inside and outside of cornfields was measured in several studies from different localities. The purpose was to obtain a representative picture of naturally occurring pollen densities to provide a perspective for laboratory and field studies of monarch larvae feeding on milkweed leaves with Bt corn pollen. Pollen density was highest (average 170.6 grains per cm2) inside the cornfield and was progressively lower from the field edge outward, falling to 14.2 grains per cm2 at 2 m. Inside the cornfield, and for each distance from the field edge, a frequency distribution is presented showing the proportion of leaf samples with different pollen densities. Inside cornfields, 95% of leaf samples had pollen densities below 600 grains per cm2 and the highest pollen density observed was 1400 grains per cm2, which occurred in a study with a rainless anthesis period. All other studies had rainfall events during the anthesis period. A single rain event can remove 54–86% of the pollen on leaves. Leaves on the upper portion of milkweed plants, where young monarch larvae tend to feed, had only 30–50% of the pollen density levels of middle leaves. In order to accurately interpret results of studies that examine the effects of Bt corn pollen on monarch butterfly larvae it is necessary to know the range and distribution of naturally occurring pollen densities on milkweed leaves. This provides a perspective on both laboratory and field studies in which monarch larvae feed on milkweed leaves with Bt corn pollen (1, 2). It lets us determine how frequently the pollen densities observed in these studies would occur in nature. The studies reported here contribute to the exposure characterization necessary for assessing the risk of Bt corn pollen to monarch butterflies. In particular, this paper describes the densities of corn pollen on milkweed leaves during corn anthesis for a number of geographic locations and under a variety of environmental conditions. We describe the pollen densities (pollen grains per cm2) that were found on leaves of milkweed plants within cornfields as well as near cornfields because corn pollen is wind-dispersed at least 60 m (3) and possibly more than 200 m (4). These data are used in a companion paper (5) on the results of laboratory studies on the responses of monarch larvae fed milkweed leaves with different densities of artificially applied Bt corn pollen. These data are also used in a second companion paper (6) to provide a frame of reference for the Bt pollen densities found in field trials of larvae feeding on milkweed leaves. Finally, these data are used in a summary companion paper (7) that provides a full risk assessment of monarchs and Bt corn pollen. In addition to characterizing naturally occurring pollen densities, we examined several factors that affect pollen deposition on milkweed leaves, including position of a leaf on the plant and rainfall.

Artigo completo disponível em http://www.pnas.org/content/98/21/11919.full

Stanley-Horn, D.; Dively, G.; Hellmich, R.; Mattila, H.; Sears, M.; Rose, R.; Jesse, L.; Losey, J.; Obrycki, J.; Lewis, L. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. *PNAS*, 98 (21): 11931-11936.

Survival and growth of monarch larvae, Danaus plexippus (L.), after exposure to either Cry1Ab-expressing pollen from three Bacillus thuringiensis (Bt) corn (Zea mays L.) events differing in toxin expression or to the insecticide, λ-cyhalothrin, were examined in field studies. First instars exposed to low doses (≈22 grains per cm2) of event-176 pollen gained 18% less weight than those exposed to Bt11 or Mon810 pollen after a 5-day exposure period. Larvae exposed to 67 pollen grains per cm2 on milkweed leaves from within an event-176 field exhibited 60% lower survivorship and 42% less weight gain compared with those exposed to leaves from outside the field. In contrast, Bt11 pollen had no effect on growth to adulthood or survival of first or third instars exposed for 5 days to ≈55 and 97 pollen grains per cm2, respectively. Similarly, no differences in larval survivorship were observed after a 4-day exposure period to leaves with 504–586 (within fields) or 18–22 (outside the field) pollen grains per cm2 collected from Bt11 and non-Bt sweet-corn fields. However, survivorship and weight gain were drastically reduced in non-Bt fields treated with λ-cyhalothrin. The effects of Bt11 and Mon810 pollen on the survivorship of larvae feeding 14 to 22 days on milkweeds in fields were negligible. Further studies should examine the lifetime and reproductive impact of Bt11 and Mon810 pollen on monarchs after long-term exposure to naturally deposited pollen.

Artigo completo disponível em http://www.pnas.org/content/98/21/11931.full

Dively, G.; Rose, R.; Sears, M.; Hellmich, R.; Stanley-Horn, D. Calvin, D.; Russo, J.; Anderson, P. 2004. Effects on monarch butterfly larvae (*Lepidoptera*: *Danaidae*) after continuous exposure to Cry1Ab expressing corn during anthesis. *Environmental Entomology*, 33: 1116-1125.

Effects on monarch butterfly, Danaus plexippus L., after continuous exposure of larvae to natural deposits of Bacillus thuringiensis (Bt) and non-Bt pollen on milkweed, were measured in five studies. First instars were exposed at 3–4 and 6–7 d after initial anthesis, either directly on milkweed plants in commercial cornfields or in the laboratory on leaves collected from milkweeds in corn plots. Pollen exposure levels ranging from 122 to 188 grains/cm2/d were similar to within-field levels that monarch butterfly populations might experience in the general population of cornfields. Results indicate that 23.7% fewer larvae exposed to these levels of Bt pollen during anthesis reached the adult stage. A risk assessment procedure used previously was updated with a simulation model estimating the proportion of second-generation monarch butterflies affected. When considered over the entire range of the Corn Belt, which represents only 50% of the breeding population, the risk to monarch butterfly larvae associated with long-term exposure to Bt corn pollen is 0.6% additional mortality. Exposure also prolonged the developmental time of larvae by 1.8 d and reduced the weights of both pupae and adults by 5.5%. The sex ratio and wing length of adults were unaffected. The ecological significance of these sublethal effects is discussed relative to generation mortality and adult performance.

Artigo completo disponível em http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1092&context=ent_pubs

4.2.2 Disseminação do milho transgênico no México

Em novembro de 2001, os cientistas americanos Quist e Chapela publicaram artigo inovador, denunciando a presença de transgenes em variedades de milho crioulo no México (centro de origem e de diversidade da espécie), enquanto o país se encontrava em estádio de moratória para uso comercial de plantas transgênicas. Esse anúncio desencadeou uma série de artigos virulentos na literatura científica. Foram criticados os pesquisadores e seus métodos, com afirmações reverberadas por diversos meios de comunicação científica, alegando a ausência de elementos suficientes para sustentação das conclusões apontadas naquele artigo. Passados sete anos, outras equipes de pesquisadores confirmaram as afirmações originais, relativamente à disseminação de transgenes no país, em áreas isoladas de plantio de milho crioulo, onde os OGMs estariam interditados. A importância do artigo original, para discussão da inevitabilidade da contaminação gênica, não foi resgatada e os prejuízos concretos, em termos do fluxo de transgenes em direção às regiões de origem, jamais serão contabilizados.

Quist, D.; Chapela, I. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature*, Vol 414.

Concerns have been raised about the potential effects of transgenic introductions on the genetic diversity of crop landraces and wild relatives in areas of crop origin and diversification, as this diversity is considered essential for global food security. Direct effects on non-target species1, 2, and the possibility of unintentionally transferring traits of ecological relevance onto landraces and wild relatives have also been sources of concern3, 4. The degree of genetic connectivity between industrial crops and their progenitors in landraces and wild relatives is a principal determinant of the evolutionary history of crops and agroecosystems throughout the world5, 6. Recent introductions of transgenic DNA constructs into agricultural fields provide unique markers to measure such connectivity. For these reasons, the detection of transgenic DNA in crop landraces is of critical importance. Here we report the presence of introgressed transgenic DNA constructs in native maize landraces grown in remote mountains in Oaxaca, Mexico, part of the Mesoamerican centre of origin and diversification of this crop.

Artigo completo disponível em http://www.artsci.wustl.edu/~anthro/bnc/quist-chapela2001.pdf

COM - Dalton, R. 2001. Transgenic corn found growing in Mexico. *Nature*, Vol 413.

Sem resumo.

http://www.nature.com/nature/journal/v413/n6854/full/413337b0.html

COM – Mann, C. 2002. Has GM corn ‘invaded’ Mexico? *Science*, Vol 295.

Sem resumo.

Artigo completo disponível em http://www.charlesmann.org/articles/Transgenic-maize-Mexico-Science1.pdf

COM - Quist, D.; Chapela, I. 2002. Quist and Chapela reply. *Nature*, Vol 416.

Sem resumo.

http://www.nature.com/nature/journal/v416/n6881/full/nature740.html

COM - Dalton, R. 2008. Modified genes spread to local maize. *Nature*, Vol 456.

Sem resumo.

Artigo completo disponível em http://www.nature.com/news/2008/081112/full/456149a.html

Piñeyro-Nelson, A.; Heerwaarden, J.; Perales, H.; Serratos-Hernandez, J.; Rangel, A.; Hufford, M.; Gepts, P.; Garay-Arroyo, A.; Rivera-Bustamante, R.; Álvarez-Buylla, E. 2009. Transgenes in Mexican maize: Molecular evidence and methodological considerations for GMO detection in landrace populations. *Molecular Ecology*, 18: 750-61.

A possible consequence of planting genetically modified organisms (GMOs) in centres of crop origin is unintended gene flow into traditional landraces. In 2001, a study reported the presence of the transgenic 35S promoter in maize landraces sampled in 2000 from the Sierra Juarez of Oaxaca, Mexico. Analysis of a large sample taken from the same region in 2003 and 2004 could not confirm the existence of transgenes, thereby casting doubt on the earlier results. These two studies were based on different sampling and analytical procedures and are thus hard to compare. Here, we present new molecular data for this region that confirm the presence of transgenes in three of 23 localities sampled in 2001. Transgene sequences were not detected in samples taken in 2002 from nine localities, while directed samples taken in 2004 from two of the positive 2001 localities were again found to contain transgenic sequences. These findings suggest the persistence or re-introduction of transgenes up until 2004 in this area. We address variability in recombinant sequence detection by analyzing the consistency of current molecular assays. We also present theoretical results on the limitations of estimating the probability of transgene detection in samples taken from landraces. The inclusion of a limited number of female gametes and, more importantly, aggregated transgene distributions may significantly lower detection probabilities. Our analytical and sampling considerations help explain discrepancies among different detection efforts, including the one presented here, and provide considerations for the establishment of monitoring protocols to detect the presence of transgenes among structured populations of landraces.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3001031/

REV - Snow, A. 2009. Unwanted transgenes re-discovered in Oaxacan maize. *Molecular Ecology*, 18: 569-571.

Transgenic [GM (genetically modified)] maize is shipped all over the world, and its ability to germinate, grow, and hybridize with local landrace s of the crop has generated tremendous scientific, social, and political controversy. The great genetic diversity of farm er-produced landraces represents a vital resource for subsistence farmers, future crop breeding, and cultural heritage preservation. Although the Mexican government banned GM maize cultivation in 1998, vast quantities of living GM grain are imported from the USA and seeds can easily enter the country by other routes. In 2000, Quist & Chapela (2001) discovered transgenes in four ears of landrace maize and in seeds from a government-sponsored Distribution Conasupo Sociedad Anonima (DICONSA) grain distribution centre in Oaxaca. Their controversial paper in Nature set off an explosion of publicity and speculation about how widely these novel genetic elements had proliferated, and what the consequences of ubiquitous gene flow might be. However, a more extensive survey of this region failed to detect transgenes in 2003 and 2004 (Ortiz-García et al. 2005a), suggesting that transgenic plants were rare or absent in the sampled fields. Here, Pineyro-Nelson et al. (2008) provide a valuable counterpoint to that survey, resolving apparent contradictions in the literature and raising the bar for subsequent studies of immigrating transgenes. They show that transgenes were present in Oaxaca in both 2001 and 2004. Their paper explains how sampling methods, statistical analyses, and problems with analytical techniques can lead to inconsistent estimates of transgene frequencies in maize populations. This is a must-read paper for those who follow genetically modified organisms (GMO) biosafety research.

http://www.ncbi.nlm.nih.gov/pubmed/19215581

4.2.3 Toxicidade associada ao consumo de milho NK603

Em novembro de 2012, Seralini e colaboradores publicaram um artigo decisivo sobre impactos toxicológicos associados ao consumo do milho NK603, levando em conta sua incorporação a dietas de médio e longo prazo, em ratos. O estudo não só apontava para riscos hepático-renais e de desregulação endócrina relevante (responsáveis pela formação de tumores), em consequência do consumo de milho tratado com o herbicida associado (fórmula comercial de Roundup), do herbicida em si e do milho GM sem herbicida (indicativo de riscos específicos da modificação genética), bem como validava a importância de se realizar estudos com duração superior a 3 meses – norma atualmente aceita pelos órgãos reguladores –, observando os primeiros sintomas graves a partir do quarto mês.

As conclusões do estudo desencadearam críticas violentas. A reação se concentrou na interpretação dos resultados e na metodologia empregada, estendendo-se a questões pessoais e à difamação dos autores e de seu instituto de pesquisa, o Centro de Pesquisa e de Informação Independente em Engenharia Genética (CRIIGEN, sigla em francês). A articulação montada com vistas a reduzir o impacto do estudo incorporou pressões sobre a revista científica que publicou o artigo, levando a alteração em seu corpo editorial. Os novos editores se retrataram, a revista minimizou as conclusões e o estudo foi acusado de deficiências não justificadas. Essa retratação gerou nova polêmica na comunidade científica – inclusive por parte de determinados autores abertamente pró-biotecnologias. Entre as fragilidades estaria o fato de as críticas se apoiarem na apresentação de "resultados inconclusivos". Considere-se, neste ponto, que esta é a condição mais frequentemente observada na literatura científica[68]. Finalmente, o estudo foi republicado em outra revista, constando novamente na base de dados da literatura científica validada.

Séralini, G-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Vendômois, J. 2012. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Food and Chemical* Toxicology, 50, 4221– 4231. Retraction in *Food and Chemical Toxicology*, 2014, 63:244.

Sem resumo.

Anúncio da retratação disponível em http://www.sciencedirect.com/science/article/pii/S0278691512005637?via=ihub

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[68] Apenas quatro motivos são oficialmente aceitos como justificativa para a retratação de um estudo científico: fraude, falta de ética, plágio ou resultados já publicados. "Resultados inconclusivos" fazem parte de rotinas incorporadas ao método, possuindo relevância expressiva nos processos de construção do conhecimento científico.

Séralini, G-E.; Mesnage, R.; Defarge, N.; Gress, S.; Hennequin, D.; Clair, E.; Malatesta, M.; Vendômois, J. 2013. Answers to critics: Why there is a long term toxicity due to a Roundup-tolerant genetically modified maize and to a Roundup herbicide. *Food and Chemical Toxicology*, 53: 476-83.

Our recent work (Séralini et al., 2012) remains to date the most detailed study involving the life-long consumption of an agricultural genetically modified organism (GMO). This is true especially for NK603 maize for which only a 90-day test for commercial release was previously conducted using the same rat strain (Hammond et al., 2004). It is also the first long term detailed research on mammals exposed to a highly diluted pesticide in its total formulation with adjuvants. This may explain why 75% of our first criticisms arising within a week, among publishing authors, come from plant biologists, some developing patents on GMOs, even if it was a toxicological paper on mammals, and from Monsanto Company who owns both the NK603 GM maize and Roundup herbicide (R). Our study has limits like any one, and here we carefully answer to all criticisms from agencies, consultants and scientists, that were sent to the Editor or to ourselves. At this level, a full debate is biased if the toxicity tests on mammals of NK603 and R obtained by Monsanto Company remain confidential and thus unavailable in an electronic format for the whole scientific community to conduct independent scrutiny of the raw data. In our article, the conclusions of long-term NK603 and Roundup toxicities came from the statistically highly discriminant findings at the biochemical level in treated groups in comparison to controls, because these findings do correspond in an blinded analysis to the pathologies observed in organs, that were in turn linked to the deaths by anatomopathologists. GM NK603 and R cannot be regarded as safe to date.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0278691512008149

Meyer, H.; Hilbeck, A. 2013. Rat feeding studies with genetically modified maize – a comparative evaluation of applied methods and risk assessment standards. *Environmental Sciences Europe*, 25:33 (doi:10.1186/2190-4715-25-33).

A 2-year rat feeding study with genetically modified NK603 maize sparked an international scientific and public debate as well as policy responses by the European Commission. The European Food Safety Authority (EFSA) evaluated the study as defective based on conceptual and methodological shortcomings by retroactive application of the recommendations of its recent guidance on 90-day feeding studies. Our comparative analysis of the three relevant NK603 publications, including a 90-day feeding study of Monsanto, showed that all of them satisfy or fail to satisfy the EFSA evaluation criteria to a comparable extent; the rejection of only one of the papers is, thus, not scientifically justified. We also show that EFSA's criteria are not standard practice in 21 other rat feeding studies lasting at a minimum of 12 months. The review reveals critical double standards in the evaluation of feeding studies submitted as proof of safety for regulatory approval to EFSA. We specifically argue that the current approach to declare statistically significant differences between genetically modified organisms and its parents as 'biologically irrelevant' based on additional reference controls lacks scientific rigor and legal justification in the European Union (EU) system. Only recently, the EU authorities started building up an implementing system based on its own legislation and supportive of the EU approach to risk assessment in the context of technology assessment. Until these issues are resolved, we do not expect that neither the public nor the scientific debate will subside.

Artigo completo disponível em http://www.enveurope.com/content/25/1/33

Heinemann, J. 2013. Food and Chemical Toxicology. *Food and Chemical Toxicology*, (53), 442.

Sem resumo.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0278691512008009

Séralini, G-E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Vendômois, J. 2014. Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26:14.

Background: The health effects of a Roundup-tolerant NK603 genetically modified (GM) maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 ppb of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in rats. This study constitutes a follow-up investigation of a 90-day feeding study conducted by Monsanto in order to obtain commercial release of this GMO, employing the same rat strain and analyzing biochemical parameters on the same number of animals per group as our investigation. Our research represents the first chronic study on these substances, in which all observations including tumors are reported chronologically. Thus, it was not designed as a carcinogenicity study. We report the major findings with 34 organs observed and 56 parameters analyzed at 11 time points for most organs.
Results: Biochemical analyses confirmed very significant chronic kidney deficiencies, for all treatments and both sexes; 76% of the altered parameters were kidney-related. In treated males, liver congestions and necrosis were 2.5 to 5.5 times higher. Marked and severe nephropathies were also generally 1.3 to 2.3 times greater. In females, all treatment groups showed a two- to threefold increase in mortality, and deaths were earlier. This difference was also evident in three male groups fed with GM maize. All results were hormone- and sex-dependent, and the pathological profiles were comparable. Females developed large mammary tumors more frequently and before controls; the pituitary was the second most disabled organ; the sex hormonal balance was modified by consumption of GM maize and Roundup treatments. Males presented up to four times more large palpable tumors starting 600 days earlier than in the control group, in which only one tumor was noted. These results may be explained by not only the non-linear endocrine-disrupting effects of Roundup but also by the overexpression of the EPSPS transgene or other mutational effects in the GM maize and their metabolic consequences.
Conclusion: Our findings imply that long-term (2 year) feeding trials need to be conducted to thoroughly evaluate the safety of GM foods and pesticides in their full commercial formulations.

Artigo completo disponível em http://www.enveurope.com/content/26/1/14

COM – Casassus, B. 2014. Paper claiming GM link with tumours republished. *Nature*, doi:10.1038/nature.2014.15463.

Sem resumo.

Artigo completo disponível em http://www.nature.com/news/paper-claiming-gm-link-with-tumours-republished-1.15463

COM - Portier, C.; Goldman, L.; Goldstein, B. 2013. Inconclusive findings: Now you see them, now you don't! *Environmental Health Perspectives*, volume 122, number 2.

Sem resumo.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3915254/

COM - Séralini, G-E.; Mesnage, R.; Defarge, N.; Vendômois, J. 2014. Conclusiveness of toxicity data and double standards. *Food and Chemical Toxicology*, doi: http://dx.doi.org/10.1016/j.fct.2014.04.018.

Sem resumo.

Artigo completo disponível em http://content.elsevierjournals.intuitiv.net/content/files/food-and-chemical-toxicology-21222339.pdf

COM - Roberfroid, M. 2014. Letter to the editor. *Food and Chemical Toxicology*, 65, 390.

Sem resumo.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0278691514000039

4.3 Conflitos de interesse e orientação da Ciência

Em paralelo às campanhas de descredibilização acima explicitadas, o tema do conflito de interesses possui também forte presença na problemática dos riscos (e suas avaliações) das plantas transgênicas para o meio ambiente e a saúde. De fato, são vários os estudos que apontam correlações positivas entre ausência de riscos das plantas transgênicas e fontes financiadoras favoráveis ao desenvolvimento da biotecnologia.

Por outro lado, pesquisadores independentes têm dificuldades em realizar estudos sobre riscos das plantas GM, já que parte significativa das informações de biossegurança tende a ser confidencial. Aqueles pesquisadores ainda precisam de autorização por parte da empresa detentora da tecnologia para usar seu material nos testes.

COM – Sharpe, V.; Gurian-Sherman, D. 2003. Competing interests. *Nature Biotechnology*, 21, 1131.

Sem resumo.

http://www.nature.com/nbt/journal/v21/n10/full/nbt1003-1131a.html

Kvakkestad, V.; Gillund, F.; Kjølberg, K.; Vatn, A. 2007. Scientists' perspectives on the deliberate release of GM crops. *Environmental Values*, 16(1): 79–104.

In this paper we analyse scientists' perspectives on the release of genetically modified (GM) crops into the environment, and the relationship between their perspectives and the context that they work within, e.g. their place of employment (university or industry), funding of their research (public or industry) and their disciplinary background (ecology, molecular biology or conventional plant breeding). We employed Q-methodology to examine these issues. Two distinct factors were identified by interviewing 62 scientists. These two factors included 92 per cent of the sample. Scientists in factor 1 had a moderately negative attitude to GM crops and emphasised the uncertainty and ignorance involved, while scientists in factor 2 had a positive attitude to GM crops and emphasised that GM crops are useful and do not represent any unique risks compared to conventional crops. Funding had a significant effect on the perspective held by the scientists in this study. No ecologists were associated with factor 2, while all the scientists employed in the GM-industry were associated with this factor. The strong effects of training and funding might justify certain institutional changes concerning how we organise science and how we make public decisions when new technologies are to be evaluated. Policy makers should encourage more interdisciplinary training and research and they should make sure that representatives of different disciplines are involved in public decisions on new technologies.

http://www.erica.demon.co.uk/EV/EV1605.html

Diels, J.; Cunha, M.; Manaia, C.; Sabugosa-Madeira, B.; Silva, M. 2011. Association of financial or professional conflict of interest to research outcomes on health risks or nutritional assessment studies of genetically modified products. *Food Policy*, 36: 197-203.

Since the first commercial cultivation of genetically modified crops in 1994, the rapidly expanding market of genetically modified seeds has given rise to a multibillion dollar industry. This fast growth, fueled by high expectations towards this new commercial technology and shareholder trust in the involved industry, has provided strong incentives for further research and development of new genetically modified plant varieties. Considering, however, the high financial stakes involved, concerns are raised over the influence that conflicts of interest may place upon articles published in peer-reviewed journals that report on health risks or nutritional value of genetically modified food products. In a study involving 94 articles selected through objective criteria, it was found that the existence of either financial or professional conflict of interest was associated to study outcomes that cast genetically modified products in a favorable light ($p = 0.005$). While financial conflict of interest alone did not correlate with research results ($p = 0.631$), a strong association was found between author affiliation to industry (professional conflict of interest) and study outcome ($p < 0.001$). We discuss these results by comparing them to similar studies on conflicts of interest in other areas, such as biomedical sciences, and hypothesize on dynamics that may help explain such connections.

http://www.sciencedirect.com/science/article/pii/S0306919210001302

REV – Domingo, J.; Bordonaba, J. 2011. A literature review on the safety assessment of genetically modified plants. *Environment International*, 37. 734-42.

In recent years, there has been a notable concern on the safety of genetically modified (GM) foods/plants, an important and complex area of research, which demands rigorous standards. Diverse groups including consumers and environmental Non Governmental Organizations (NGO) have suggested that all GM foods/plants should be subjected to long-term animal feeding studies before approval for human consumption. In 2000 and 2006, we reviewed the information published in international scientific journals, noting that the number of references concerning human and animal toxicological/health risks studies on GM foods/plants was very limited. The main goal of the present review was to assess the current state-of-the-art regarding the potential adverse effects/safety assessment of GM plants for human consumption. The number of citations found in databases (PubMed and Scopus) has dramatically increased since 2006. However, new information on products such as potatoes, cucumber, peas or tomatoes, among others was not available. Corn/maize, rice, and soybeans were included in the present review. An equilibrium in the number research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was currently observed. Nevertheless, it should be noted that most of these studies have been conducted by biotechnology companies responsible of commercializing these GM plants. These findings suggest a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies. All this recent information is herein critically reviewed.

http://www.ncbi.nlm.nih.gov/pubmed/21296423

Séralini, G-E.; Mesnage, R.; Defarge, N.; Gress, S.; Hennequin, D.; Clair, E.; Malatesta, M.; Vendômois, J. 2013. Answers to critics: Why there is a long term toxicity due to Roundup-tolerant genetically modified maize and to a Roundup herbicide. *Food and Chemical Toxicology*, 53: 476-83.

Our recent work (Séralini et al., 2012) remains to date the most detailed study involving the life-long consumption of an agricultural genetically modified organism (GMO). This is true especially for NK603 maize for which only a 90-day test for commercial release was previously conducted using the same rat strain (Hammond et al., 2004). It is also the first long term detailed research on mammals exposed to a highly diluted pesticide in its total formulation with adjuvants. This may explain why 75% of our first criticisms arising within a week, among publishing authors, come from plant biologists, some developing patents on GMOs, even if it was a toxicological paper on mammals, and from Monsanto Company who owns both the NK603 GM maize and Roundup herbicide (R). Our study has limits like any one, and here we carefully answer to all criticisms from agencies, consultants and scientists, that were sent to the Editor or to ourselves. At this level, a full debate is biased if the toxicity tests on mammals of NK603 and R obtained by Monsanto Company remain confidential and thus unavailable in an electronic format for the whole scientific community to conduct independent scrutiny of the raw data. In our article, the conclusions of long-term NK603 and Roundup toxicities came from the statistically highly discriminant findings at the biochemical level in treated groups in comparison to controls, because these findings do correspond in an blinded analysis to the pathologies observed in organs, that were in turn linked to the deaths by anatomopathologists. GM NK603 and R cannot be regarded as safe to date.

Artigo completo disponível em http://www.sciencedirect.com/science/article/pii/S0278691512008149

Nielsen, K. 2013. Biosafety data as confidential business information. *PLOS Biology*, 11(3): e1001499.

Confidential business information (CBI) is a necessary tool to protect commercial interests in the rapidly developing field of gene technology. CBI is also often claimed for documentation and materials supporting the biosafety assessments of genetically modified organisms (GMOs) intended for environmental release, food, and feed use. However, such claims oftentimes marginally serve their legitimate purpose to protect commercial interests and unnecessarily limit transparency and public peer review of data submitted to regulatory authorities. CBI and proprietary claims also restrict access to transgene sequence data, transgenic seeds, and other GMO materials, which precludes the development of independent research and monitoring strategies. In the long run, such claims are counterproductive to the safe and responsible commercial development of GM technology as they hinder the accumulation of biosafety data in the open, peer-reviewed literature, which is needed for both public and scientific consensus-building on safety issues and for improvements to the risk-assessment procedure itself. The increasing recognition of conflicts of interest as an invariable part of market-oriented safety-data production, interpretation, and risk communication also calls for transparency and open access to safety-related data and assessments.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3589341/pdf/pbio.1001499.pdf

5 Embates sociais e segurança alimentar

A ausência de consenso científico extrapola o tema dos riscos biológicos relacionados à expansão das PGMs, sobre o meio ambiente e a saúde humana. Questões como custos e benefícios, oportunidade e conveniência para a sociedade e seus grupos populacionais também são objetos de disputa. O tema da rentabilidade econômica para diferentes perfis de agricultores bem como a questão das vantagens prometidas pelas indústrias em seu contraste com dados de realidade alimentam as polêmicas. Entre as afirmativas mais reiteradas, no foco das disputas, estão as hipóteses de essencialidade das PGMs, para a luta global contra a fome (incorporando desdobramentos políticos relativos a segurança e soberania alimentar), e a pertinência do desenvolvimento de plantas biofortificadas por meio da engenharia genética.

Os artigos a seguir apontam realidade oposta àquela prometida pelo setor da biotecnologia. Suavizando e relativizando os supostos benefícios dessas tecnologias, sobre todas as dimensões referidas, a bibliografia destaca a superioridade do melhoramento convencional e dos sistemas de base agroecológica para se enfrentar o problema da fome no mundo.

Para tratar desses temas, fazem-se necessárias reflexões sobre o papel dos agricultores na construção de demandas e na orientação de pesquisas agrícolas. O Sistema oficial de pesquisas, patrocinado por recursos da sociedade, parece afastar-se de suas finalidades, atendendo a interesses que respondem não ao tema do desenvolvimento nacional, mas, sim, a objetivos estratégicos das indústrias de biotecnologia e seus associados.

Cavalli, S. 2001. Segurança alimentar: a abordagem dos alimentos transgênicos. *Revista de Nutrição da Puccamp*, 14 (suplemento): 41-46.

O objetivo desta comunicação é discutir a relação entre a segurança alimentar e os alimentos geneticamente modificados. A biotecnologia e a engenharia genética têm sido encaradas como parte da segunda revolução verde, justificando-se, entre outras prerrogativas, o uso de alimentos transgênicos como solução do problema da fome no mundo, sem risco à saúde da população e ao meio ambiente. Face a essa premissa, discute-se a segurança alimentar sob os enfoques qualitativos e quantitativos, destacando as atribuições dos órgãos responsáveis e suas interfaces com alimentos geneticamente modificados. Acredita-se que os alimentos transgênicos não sejam a solução para o problema da fome no mundo.

Artigo completo disponível em http://www.scielo.br/scielo.php?pid=S1415-52732001000400007&script=sci_arttext

Tucker, G. 2003. Nutritional enhancement of plants. *Current Opinion in Biotechnology*, 14:221–225.

Plants can provide most of the nutrients required in the human diet; however, the major staple crops are often deficient in some of these nutrients. Thus, malnutrition, with respect to micronutrients like vitamin A, iron and zinc, affects >40% of the world's population. Advances in molecular biology are being exploited to produce crops enhanced in these key nutrients. Other nutritional targets include the modification of fatty acid composition and the enhancement of antioxidant levels, particularly carotenoids, such as lycopene, and flavonoids. However, the benefit of these 'biofortified' crops to human nutrition remains to be elucidated.

http://www.ncbi.nlm.nih.gov/pubmed/12732325

Lacey, H. 2007. Há alternativas ao uso dos transgênicos? *Novos estudos – CEBRAP*, no.78, São Paulo.

A existência ou não de alternativa ao uso de transgênicos capaz de satisfazer a demanda mundial por alimento e nutrientes é uma questão que permanece aberta à investigação científica. A importância dos transgênicos ainda não está bem fundamentada no conhecimento científico disponível, em parte porque as conquistas e o potencial da agroecologia não foram objeto de atenção científica suficiente.

Artigo completo disponível em http://www.scielo.br/scielo.php?pid=S0101-33002007000200005&script=sci_arttext

COM - Herren, H. 2008. Food fault lines. *Nature*, 456, 21.

Sem resumo.

Artigo completo disponível em http://www.nature.com/nature/journal/v456/n1s/pdf/twas08.21a.pdf

Richards, P.; Bruin-Hoekzema, M.; Hughes, S.; Kudadjie-Freeman, C.; Offei, S.; Struik, P.; Zannou, A. 2009. Seed systems for African food security: linking molecular genetic analysis and cultivator knowledge in West Africa. *Int J Technol Manage*, 45:196–214.

A challenge for African countries is how to integrate new sources of knowledge on plant genetics with knowledge from farmer practice to help improve food security. This paper considers the knowledge content of farmer seed systems in the light of a distinction drawn in artificial intelligence research between supervised and unsupervised learning. Supervised learning applied to seed systems performance has a poor record in Africa. The paper discusses an alternative – unsupervised learning supported by functional genomic analysis. Recent work in West Africa on sorghum, African rice and white yam is described. Requirements for laboratory-based analytical support are outlined. A science-backed 'farmer first' approach – while feasible – will require a shift in policy and funding by major investors.

http://www.inderscience.com/info/inarticle.php?artid=21528

Vanloqueren, G.; Baret, P. 2009. How agricultural research systems shape a technological regime that develops genetic engineering but locks out agroecological innovations. *Research Policy*, 39: 971–83.

Agricultural science and technology (S&T) is under great scrutiny. Reorientation towards more holistic approaches, including agroecology, has recently been backed by a global international assessment of agriculture S&T for development (IAASTD). Understanding the past and current trends of agricultural S&T is crucial if such recommendations are to be implemented. This paper shows how the concepts of technological paradigms and trajectories can help analyse the agricultural S&T landscape and dynamics. Genetic engineering and agroecology can be usefully analysed as two different technological paradigms, even though they have not been equally successful in influencing agricultural research. We used a Systems of Innovation (SI) approach to identify the determinants of innovation (the factors that influence research choices) within agricultural research systems. The influence of each determinant is systematically described (e.g. funding priorities, scientists' cognitive and cultural routines etc.). As a result of their interactions, these determinants construct a technological regime and a lock-in situation that hinders the development of agroecological engineering. Issues linked to breaking out of this lock-in situation are finally discussed.

http://www.sciencedirect.com/science/article/pii/S0048733309000614

Howard, P. 2009. Visualizing consolidation in the global seed industry: 1996–2008. *Sustainability*, 1, 1266-1287; doi:10.3390/su1041266.

The commercial seed industry has undergone tremendous consolidation in the last 40 years as transnational corporations entered this agricultural sector, and acquired or merged with competing firms. This trend is associated with impacts that constrain the opportunities for renewable agriculture, such as reductions in seed lines and a declining prevalence of seed saving. To better characterize the current structure of the industry, ownership changes from 1996 to 2008 are represented visually with information graphics. Since the commercialization of transgenic crops in the mid-1990s, the sale of seeds has become dominated globally by Monsanto, DuPont and Syngenta. In addition, the largest firms are increasingly networked through agreements to cross-license transgenic seed traits.

Artigo completo disponível em http://www.mdpi.com/2071-1050/1/4/1266

REV - Stone, G. 2010. The anthropology of genetically modified crops. *Annual Review of Anthropology*, 39: 381– 400.

By late in the twentieth century, scientists had succeeded in manipulating organisms at the genetic level, mainly by gene transfer. The major impact of this technology has been seen in the spread of genetically modified (GM) crops, which has occurred with little controversy in some areas and with fierce controversy elsewhere. GM crops raise a very wide range of questions, and I address three areas of particular interest for anthropology and its allied fields. First are the political economic aspects of GM, which include patenting of life forms and new relationships among agriculture, industry, and the academy. Second is the wide diversity in response and resistance to the technology. Third is the much-debated question of GM crops for the developing world. This analysis is approached first by determining what controls research agendas and then by evaluating actual impacts of crops to date.

Artigo completo disponível em http://artsci.wustl.edu/~anthro/research/stone/stone-annualreview-2010.pdf

COM – Tirado, R.; Johnston, P. 2010. Food security: GM crops threaten biodiversity. *Science*, 328(5975):170-1. doi: 10.1126/science.328.5975.170-a.

Sem resumo.

http://www.ncbi.nlm.nih.gov/pubmed/20378798

Stone, G.; Glover, D. 2011. Genetically modified crops and the 'food crisis': discourse and material impacts. *Development in Practice*, 21:4-5, 509-516.

Uma onda de reportagens na mídia e argumentações retóricas apontaram as culturas geneticamente modificadas como uma solução para a "crise alimentar global" que se manifestou com o aumento repentino nos preços mundiais dos alimentos durante 2007–08. Argumentava-se em geral sobre o potencial das tecnologias GM de lidar com a crise, mesmo embora as culturas e características úteis tipicamente invocadas tivessem ainda de ser desenvolvidas e apesar do fato do progresso real ter sido alcançado na verdade utilizando-se cultivos convencionais. O caso ilustra vividamente o uso instrumental da retórica da crise alimentar para promover culturas GM.

Artigo completo disponível em http://www.artsci.wustl.edu/~anthro/research/stone/Stone_Glover_2011.pdf

Henn, M. 2011. The speculator's bread: What is behind rising food prices? *EMBO Reports*, 12(4): 296–301.

Speculators increasingly invest into food markets for financial gain, with potentially devastating consequences for millions of poor people who cannot afford food at inflated prices.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3077254/

Birch, A.; Begg, G.; Squire, G. 2011. How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. *Journal of Experimental Botany*, 62, *3251-3261.*

Drivers behind food security and crop protection issues are discussed in relation to food losses caused by pests. Pests globally consume food estimated to feed an additional one billion people. Key drivers include rapid human population increase, climate change, loss of beneficial on-farm biodiversity, reduction in per capita cropped land, water shortages, and EU pesticide withdrawals under policies relating to 91/414 EEC. IPM (Integrated Pest Management) will be compulsory for all EU agriculture by 2014 and is also being widely adopted globally. IPM offers a 'toolbox' of complementary crop- and region-specific crop protection solutions to address these rising pressures. IPM aims for more sustainable solutions by using complementary technologies. The applied research challenge now is to reduce selection pressure on single solution strategies, by creating additive/synergistic interactions between IPM components. IPM is compatible with organic, conventional, and GM cropping systems and is flexible, allowing regional fine-tuning. It reduces pests below economic thresholds utilizing key 'ecological services', particularly biocontrol. A recent global review demonstrates that IPM can reduce pesticide use and increase yields of most of the major crops studied. Landscape scale 'ecological engineering', together with genetic improvement of new crop varieties, will enhance the durability of pest-resistant cultivars (conventional and GM). IPM will also promote compatibility with semiochemicals, biopesticides, precision pest monitoring tools, and rapid diagnostics. These combined strategies are urgently needed and are best achieved via multi-disciplinary research, including complex spatio-temporal modelling at farm and landscape scales. Integrative and synergistic use of existing and new IPM technologies will help meet future food production needs more sustainably in developed and developing countries, in an era of reduced pesticide availability. Current IPM research gaps are identified and discussed.

Artigo completo disponível em http://jxb.oxfordjournals.org/content/early/2011/06/08/jxb.err064.full.pdf+html

Jacobsen, S-E.; Sørensen, M.; Pedersen, S.; Weiner, J. 2013. Feeding the world: genetically modified crops versus agricultural biodiversity. *Agronomy for Sustainable Development*, Volume 33, Issue 4, pp 651-662.

The growing demand for food poses major challenges to humankind. We have to safeguard both biodiversity and arable land for future agricultural food production, and we need to protect genetic diversity to safeguard ecosystem resilience. We must produce more food with less input, while deploying every effort to minimize risk. Agricultural sustainability is no longer optional but mandatory. There is still an on-going debate among researchers and in the media on the best strategy to keep pace with global population growth and increasing food demand. One strategy favors the use of genetically modified (GM) crops, while another strategy focuses on agricultural biodiversity. Here, we discuss two obstacles to sustainable agriculture solutions. The first obstacle is the claim that genetically modified crops are necessary if we are to secure food production within the next decades. This claim has no scientific support, but is rather a reflection of corporate interests. The second obstacle is the resultant shortage of research funds for agrobiodiversity solutions in comparison with funding for research in genetic modification of crops. Favoring biodiversity does not exclude any future biotechnological contributions, but favoring biotechnology threatens future biodiversity resources. An objective review of current knowledge places GM crops far down the list of potential solutions in the coming decades. We conclude that much of the research funding currently available for the development of GM crops would be much better spent in other research areas of plant science, e.g., nutrition, policy research, governance, and solutions close to local market conditions if the goal is to provide sufficient food for the world's growing population in a sustainable way.

Artigo completo disponível em http://www.ensser.org/fileadmin/files/2013_Jacobsen-etal.pdf

Longe de pretender fornecer uma lista exaustiva dos estudos e dos resultados obtidos, por meio de melhoramento convencional, para a geração de plantas biofortificadas e de novas variedades adaptadas aos desafios agronômicos e ambientais nestes tempos de acelerada mudança climática (notadamente para o caso de sistemas de base agroecológica), referenciamos abaixo alguns casos concretos e discussões pertinentes sobre o tema.

REV - Pretty, J.; Morison, J.; Hine, R. 2003. Reducing food poverty by increasing agricultural sustainability in developing countries. *Agriculture, Ecosystems & Environment*; Volume 95, Issue 1, Pages 217–234.

We examined the extent to which farmers have improved food production in recent years with low cost, locally available and environmentally sensitive practices and technologies. We analysed by survey during 1999–2000 208 projects in 52 developing countries, in which 8.98 million farmers have adopted these practices and technologies on 28.92 million hectares, representing 3.0% of the 960 million hectares of arable and permanent crops in Africa, Asia and Latin America. We found improvements in food production occurring through one or more of four mechanisms: (i) intensification of a single component of farm system; (ii) addition of a new productive element to a farm system; (iii) better use of water and land, so increasing cropping intensity; (iv) improvements in per hectare yields of staples through introduction of new regenerative elements into farm systems and new locally appropriate crop varieties and animal breeds. The 89 projects with reliable yield data show an average per project increase in per hectare food production of 93%. The weighted average increases across these projects were 37% per farm and 48% per hectare. In the 80 projects with small (<5 ha) farms where cereals were the main staples, the 4.42 million farms on 3.58 million hectares increased household food production by 1.71 t per year. We report on the practices and technologies that have led to these increases: increased water use efficiency, improvements to soil health and fertility, and pest control with minimal or zero-pesticide use. This research reveals promising advances in the adoption of practices and technologies that are likely to be more sustainable, with substantial benefits for the rural poor. With further explicit support, particularly through national policy reforms and better markets, these improvements in food security could spread to much larger numbers of farmers and rural people in the coming decades.

http://www.sciencedirect.com/science/article/pii/S0167880902000877

Zhu, Y.; Chen, H.; Fan, J.; Wang, Y.; Li, Y.; Chen, J.; Fan, J.; Yang, S.; Hu, L.; Leung, H.; Mew, T.; Teng, P.; Wang, Z.; Mundt, C. 2000. Genetic diversity and disease control in rice. *Nature*, 17, 406: 718–722.

Crop heterogeneity is a possible solution to the vulnerability of monocultured crops to disease. Both theory and observation indicate that genetic heterogeneity provides greater disease suppression when used over large areas, though experimental data are lacking. Here we report a unique cooperation among farmers, researchers and extension personnel in Yunnan Province, China—genetically diversified rice crops were planted in all the rice fields in five townships in 1998 and ten townships in 1999. Control plots of monocultured crops allowed us to calculate the effect of diversity on the severity of rice blast, the major disease of rice. Disease-susceptible rice varieties planted in mixtures with resistant varieties had 89% greater yield and blast was 94% less severe than when they were grown in monoculture. The experiment was so successful that fungicidal sprays were no longer applied by the end of the two-year programme. Our results support the view that intraspecific crop diversification provides an ecological approach to disease control that can be highly effective over a large area and contribute to the sustainability of crop production.

http://www.ncbi.nlm.nih.gov/pubmed/10963595

COM – Xu, K.; Xu, X.; Fukao, T.; Canlas, P.; Maghirang-Rodriguez, R.; Heuer, S.; Ismail, A.; Bailey-Serres, J.; Ronald, P.; Mackill, D. 2006. *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature*, 442(7103): 705-708.

Sem resumo.

http://www.nature.com/nature/journal/v442/n7103/full/nature04920.html

Virk, D.; Steele, K.; Witcomb, J. 2007. Mass and line selection can produce equally uniform rice varieties. *Field Crops Research*, 100 (2–3): 341–7.

We compared intra-varietal variation of two rice varieties (Ashoka 200F and Ashoka 900F) produced by a very simple bulk breeding method (mass selection with no line selection at any stage) with one line-selected variety (Ashoka 228), all derived from the same cross. Their parents, the upland variety Kalinga III and the irrigated transplanted medium-lowland variety IR64, both originated through line selection and were used as control varieties. Panicle-to-row progenies of all the varieties were evaluated in eastern India under irrigation in the dry season and in the rainy season using two water regimes; entirely rainfed or with supplementary irrigation. Intra-varietal variation for quantitative traits, irrespective of the method of breeding, was low. Only Ashoka 200F had significant variation for grain yield, days to flowering and plant height but only in the dry season. This season was not the target of the breeding programme and selection would be unlikely to produce a worthwhile response for the rainy season. At 43 SSR loci there was more genetic variation between lines within the bulk-selected varieties than within Ashoka 228. Kalinga III was the least variable variety while IR64 had less heterozygosity but greater heterogeneity than the two bulk-selected varieties. Despite the greater simplicity of the method, mass selection in bulk populations produced varieties that met the distinctness, uniformity and stability (DUS) criteria for seed certification in India. Such uniformity was achieved because of the high selection pressures applied to the bulk. We conclude that this very simple bulk-population breeding approach is highly cost-effective and produces sufficient seed for wide testing earlier than any alternative method.

http://www.sciencedirect.com/science/article/pii/S0378429006001821

Collard, B.; Mackill, D. 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491): 557-572.

DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS). The large number of quantitative trait loci (QTLs) mapping studies for diverse crops species have provided an abundance of DNA marker-trait associations. In this review, we present an overview of the advantages of MAS and its most widely used applications in plant breeding, providing examples from cereal crops. We also consider reasons why MAS has had only a small impact on plant breeding so far and suggest ways in which the potential of MAS can be realized. Finally, we discuss reasons why the greater adoption of MAS in the future is inevitable, although the extent of its use will depend on available resources, especially for orphan crops, and may be delayed in less-developed countries. Achieving a substantial impact on crop improvement by MAS represents the great challenge for agricultural scientists in the next few decades.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2610170/

Singh, A.; Ansari, M.; Pareek, A.; Singla-Pareek, S. 2008. Raising salinity tolerant rice: Recent progress and future perspectives. *Physiology and Molecular Biology of Plants*, 14: 23 – 32.

With the rapid growth in population consuming rice as staple food and the deteriorating soil and water quality around the globe, there is an urgent need to understand the response of this important crop towards these environmental abuses. With the ultimate goal to raise rice plant with better suitability towards rapidly changing environmental inputs, intensive efforts are on worldwide employing physiological, biochemical and molecular tools to perform this task. In this regard, efforts of plant breeders need to be duly acknowledged as several salinity tolerant varieties have reached the farmers field. Parallel efforts from molecular biologists have yielded relevant knowledge related to perturbations in gene expression and proteins during stress. Employing transgenic technology, functional validation of various target genes involved in diverse processes such as signaling, transcription, ion homeostasis, antioxidant defense etc for enhanced salinity stress tolerance has been attempted in various model systems and some of them have been extended to crop plant rice too. However, the fact remains that these transgenic plants showing improved performance towards salinity stress are yet to move from 'lab to the land'. Pondering this, we propose that future efforts should be channelized more towards multigene engineering that may enable the taming of this multigene controlled trait. Recent technological achievements such as the whole genome sequencing of rice is leading to a shift from single gene based studies to genome wide analysis that may prove to be a boon in re-defining salt stress responsive targets.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3550660/

Hattori, Y.; Nagai, K.; Furukawa, S.; Song, X-J.; Kawano, R.; Sakakibara, H.; Wu, J.; Matsumoto, T.; Yoshimura, A.; Kitano, H.; Matsuoka, M.; Mori, H.; Ashikari, M. 2009. The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. *Nature*, 460: 1026–1030.

Living organisms must acquire new biological functions to adapt to changing and hostile environments. Deepwater rice has evolved and adapted to flooding by acquiring the ability to significantly elongate its internodes, which have hollow structures and function as snorkels to allow gas exchange with the atmosphere, and thus prevent drowning1, 2, 3. Many physiological studies have shown that the phytohormones ethylene, gibberellin and abscisic acid are involved in this response4, 5, 6, 7, 8, but the gene(s) responsible for this trait has not been identified. Here we show the molecular mechanism of deepwater response through the identification of the genes SNORKEL1 and SNORKEL2, which trigger deepwater response by encoding ethylene response factors involved in ethylene signalling. Under deepwater conditions, ethylene accumulates in the plant and induces expression of these two genes. The products of SNORKEL1 and SNORKEL2 then trigger remarkable internode elongation via gibberellin. We also demonstrate that the introduction of three quantitative trait loci from deepwater rice into non-deepwater rice enabled the latter to become deepwater rice. This discovery will contribute to rice breeding in lowland areas that are frequently flooded during the rainy season.

http://www.nature.com/nature/journal/v460/n7258/abs/nature08258.html

Li, S.; Nugroho, A.; Rocheford, T.; White, W. 2010. Vitamin A equivalence of the β-carotene in β-carotene–biofortified maize porridge consumed by women. A*merican Journal of Clinical Nutrition*.

Background: β-Carotene-biofortified maize is being developed through plant breeding as a sustainable agronomic approach to alleviate vitamin A deficiency.
Objective: Our objective was to quantify the vitamin A equivalence of the β-carotene in β-carotene-biofortified maize based on consumption of a single serving of maize porridge.

Design: Six healthy women each consumed three 250-g portions of maize porridge as follows: 1) β-carotene-biofortified maize porridge containing 527 μg (0.98 μmol) total β-carotene, 2) white maize porridge with a β-carotene reference dose containing 595 μg (1.11 μmol) added β-carotene, and 3) white maize porridge with a vitamin A reference dose containing 286 μg retinol activity equivelent (1.00 μmol) added retinyl palmitate. Each portion contained 8.0 g added sunflower oil. The porridges were consumed in random order separated by ≥2 wk. Blood samples were collected over 9 h. Retinyl palmitate was analyzed in plasma triacylglycerol-rich lipoprotein (TRL) fractions by HPLC with coulometric array electrochemical detection.
Results: Mean (± SD) areas under the curve for retinyl palmitate in the TRL fractions (nmol · h) were 24.0 ± 9.4, 89.7 ± 34.7, and 80.1 ± 24.8 after ingestion of the β-carotene-biofortified maize porridge, the white maize porridge with the β-carotene reference dose, and the white maize porridge with the vitamin A reference dose, respectively. On average, 6.48 ± 3.51 μg (mean ± SD) of the β-carotene in β-carotene-biofortified maize porridge and 2.34 ± 1.61 μg of the β-carotene in the reference dose were each equivalent to 1 μg retinol.
Conclusion: β-Carotene in biofortified maize has good bioavailability as a plant source of vitamin A.

Artigo completo disponível em http://ajcn.nutrition.org/content/92/5/1105.long

COM - Gilbert, N. 2014. Cross-bred crops get fit faster. *Nature*, 292, Vol 513.

Sem resumo.

Artigo completo disponível em http://www.nature.com/news/cross-bred-crops-get-fit-faster-1.15940.

Considerações finais

Essa revisão dos estudos e artigos publicados na literatura científica que aponta para riscos e incertezas sobre o uso de plantas transgênicas em escala comercial não tem a pretensão de ser exaustiva. Ainda assim, reunindo cerca de 750 documentos que contrariam em determinados aspectos a visão dominante na grande mídia e nas agências reguladoras, fornece prova irrefutável de ausência de consenso da comunidade científica sobre o tema.

Cabe esclarecer o fato de que essa publicação não pretende apoiar ou reafirmar os achados e as conclusões apresentados nesses artigos. As hipóteses de riscos, ainda que reiteradas, não atestam a existência de problemas reais, assim como no extremo oposto, afirmativas repetidas de ausência de riscos, baseadas em estudos inconclusivos, não oferecem segurança efetiva ao consumidor.

O objetivo deste documento é comprovar um fato simples: revisão extensiva de estudos científicos publicados em revistas especializadas permite afirmar que, no limite do conhecimento científico atual, não é possível concluir pela ausência de riscos ao plantio e ao consumo de plantas transgênicas e suas partes. Ou, resumidamente, não é possível afirmar, com seriedade e isenção, que a tecnologia envolvida não implique riscos relevantes para a saúde e o ambiente.

Ademais, esse livro ressalta a necessidade de trabalho similar aplicado à sistematização de estudos publicados na literatura científica e que concluem pela ausência de riscos e incertezas associados ao uso de plantas transgênicas em escala comercial. Tal pesquisa permitiria qualificar e quantificar melhor a polêmica que ocorre na comunidade científica sobre os temas referentes à biossegurança de tais plantas. Destaque-se, neste ponto, que os documentos utilizados pelas agências reguladoras na avaliação de riscos associados às plantas transgênicas, são, predominantemente, produzidos, financiados ou apoiados pelas empresas interessadas ou por pesquisadores que fazem ou fizeram parte de seus quadros funcionais.

Em paralelo, a análise das datas de publicação desses estudos também mostra que a polêmica continua intensa e atual, refutando alegações falaciosas de se tratar de debate resolvido e ultrapassado.

De fato, desde a liberação comercial da primeira planta transgênica para plantio e consumo – o tomate Flav/Savr em 1994 – os debates não arrefeceram. Ao contrário, tornaram-se mais acirrados, expandindo-se para todos os campos da biossegurança e afetando os pressupostos mais fundamentais da engenharia genética. Atualmente, por paradoxal que possa parecer, os geneticistas recusam tentativas de definição para o que seja um gene. Sabe-se, com segurança, que o conhecimento científico atual não é robusto o suficiente para definir, com precisão, o que seja um gene.

Além da expansão do campo das incertezas, como se percebe neste trabalho, acumulam-se registros de impactos negativos, concretos, decorrentes do uso dessa tecnologia. Eles tendem a ser mais graves nos países que mais precocemente adotaram as plantas transgênicas numa escala espaço-temporal significativa, mas estão presentes em todos os locais. Tal afirmação pode ser ilustrada, entre outros fatos, pelo aumento do uso de pesticidas – devido notadamente ao desenvolvimento de populações de plantas e insetos geneticamente resistentes à tecnologia e aos problemas de manejo dos agrossistemas – em razão da disseminação de transgenes em espécies agrícolas e ruderais.

Ainda não é possível prever claramente, nem tampouco descartar de modo taxativo os impactos negativos associados ao consumo de OGMs, embora já estejam disponíveis mais de dez estudos que apontam de forma conclusiva para efeitos tóxicos, notadamente hepático-renais, associados ao consumo de plantas transgênicas já liberadas comercialmente. Esses resultados apareceriam no médio e no longo prazo sempre que tais plantas viessem a representar parte significativa da dieta quotidiana dos organismos sob teste.

As informações contidas nesta publicação e, consequentemente, em todos os artigos científicos aqui resumidos contrastam com o posicionamento dos órgãos de avaliação do risco de tais plantas. Uma vez que, tanto no Brasil (CTNBio) como na maioria dos países onde essa tecnologia prospera (FSANZ, GTR, EFSA, entre outros), aquelas organizações reguladoras deveriam considerar a bibliografia científica atualizada, surpreende que os cerca de 750 artigos aqui citados permaneçam no limbo da invisibilidade.

Ao tomar decisões ou emitir opiniões supostamente isentas, mas negando espaço para as dúvidas aqui colocadas, as agências reguladoras permitem questionamentos que extrapolam a questão científica. Não é irrelevante o fato de que, por maioria de seus membros, aquelas agências sempre se posicionem favoravelmente ao uso das plantas transgênicas para cultivo e consumo humano e animal.

Tamanho acervo de estudos incorporados à literatura científica – representando a opinião de cerca de MILHARES de pesquisadores com estudos aceitos por revistas especializadas – evidencia a importância do viés político e das pressões econômicas subjacentes ao posicionamento dos diferentes países quando decidem liberar comercialmente tais plantas. De fato, a Ciência com um todo, e a biogenética em particular, não teria por si só a competência – ou a legitimidade – para decidir se os riscos ilustrados nesse documento devem ser considerados aceitáveis ou negligenciáveis pela sociedade como um todo. Nesse sentido, faz-se necessário um chamado ao protagonismo social, envolvendo também o conhecimento científico acumulado em outras áreas do saber atual.

Além disso, urge a revisão do regulamento decisório onde comissões ou agências compostas apenas por cientistas opinam em caráter finalístico sobre a disseminação de transgenes (e/ou seus produtos de expressão – conhecidos ou não) no meio ambiente e nas cadeias alimentares humanas e animais. Evidentemente, esse é o caso do Brasil onde, por meio da CTNBio, 27 pessoas decidem sobre riscos que ameaçam o futuro da biodiversidade nacional.

Na opinião dos autores deste documento, as informações aqui contidas, recolhidas do acervo publicado pela comunidade científica internacional, justificam decisão política pela moratória ao cultivo e ao consumo das plantas transgênicas, principalmente Bt e TH. A simples aplicação do Princípio da Precaução, levando em conta este acervo, não permitiria conclusão oposta.

Nesse contexto, cabe lembrar que essa opção já foi adotada por cerca de 20 nações – de modo temporário ou definitivo – e que plantas transgênicas são hoje cultivadas em escala significativa somente em menos de dez países no mundo.

Os estudos referenciados nesta publicação contrastam fortemente com afirmações repetitivas da indústria de biotecnologias agrícolas e de agroquímicos, muitas vezes literalmente incorporadas a pareceres favoráveis apresentados na CTNBio, que comunicam a suposta ausência de riscos para o meio ambiente e a saúde pública de seus produtos, de maneira a criar ilusão de posição unânime da comunidade científica sobre o tema.

No que diz respeito especificamente à problemática dos riscos para a saúde associados ao consumo de plantas transgênicas, vale comentar nesta conclusão o argumento sistemático usado por essas partes quando confrontadas com artigos que apontam tais biorriscos: “alimentos transgênicos já são consumidos desde mais de 15 anos ao redor do mundo e nunca foram observados problemas de saúde pública”. No limite do conhecimento desses autores, não há na literatura científica um único estudo epidemiológico que sustente tal afirmação. Entretanto, esse documento referenciou pelo menos três artigos[69] que concluem, ao contrário, que o consumo no longo prazo de produtos geneticamente modificados pode gerar uma degradação geral da saúde pública e, em especial, dos consu-

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[69] Os dois outros artigos não explicitados aqui dizem respeito aos estudos de Swamson et al. (2014), disponível na Parte 4, e de Seneff et al. (2015), disponível no posfácio.

midores. Um deles objetivava caracterizar fatores dietéticos envolvidos na epidemia de obesidade da população dos EUA (Shao & Chin, 2011[70]). Tais autores afirmam que:

> Consequentemente, fomos capazes de demonstrar uma ligação nova entre o consumo de produtos à base de milho e o aumento da tendência à obesidade que nunca antes foi atribuída à epidemia de obesidade. Essa correlação coincide com a introdução de milhos transgênicos na cadeia alimentar humana, o que levanta então uma nova hipótese que deveria ser testada em modelos moleculares e animais da obesidade[71]. (SHAO; CHIN, 2011, p. 253)

Coincidentemente com o fechamento desta publicação, um novo estudo foi publicado na literatura científica com o título de “*No scientific consensus on GMOs safety*”. Endossado por mais de 300 investigadores independentes oriundos de diversos países, Hilbeck et al. (2015)[72] afirmam que a literatura científica disponível, publicada em revistas especializadas, não sustenta a tão divulgada hipótese de inocuidade dos OGMs. Conduzindo linha de pesquisa similar a adotada neste livro, aqueles autores afirmam que a escassez e a contradição de resultados disponíveis na literatura científica impedem qualquer afirmativa responsavelmente sustentada por evidências de pesquisas. Assim, esforços para atestar ou veicular a ideia de segurança (ou mesmo de insegurança) dos transgênicos seriam cientificamente incorretos na medida em que não se apoiariam em análise objetiva da literatura científica atualizada.

Finalmente, resta agradecer a todos os cientistas que pesquisam e que publicam sobre a biossegurança das plantas transgênicas. Apesar da conjuntura político-ideológica dificultar estudos que

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[70] Qin Shao and Khew-Voon Chin (2011): *Survey of American food trends and the growing obesity epidemic. Nutr Res Pract.* 5(3): 253-259.

[71] No texto original: “Therefore, we were able to demonstrate a novel link between the consumption of corn products and rising obesity trends that has not been previously attributed to the obesity epidemic. This correlation coincides with the introduction of bioengineered corns into the human food chain, thus raising a new hypothesis that should be tested in molecular and animal models of obesity.”

[72] Angelika Hilbeck, Rosa Binimelis, Nicolas Defarge, Ricarda Steinbrecher, András Székács, Fern Wickson, Michael Antoniou, Philip L. Bereano, Ethel Ann Clark, Michael Hansen, Eva Novotny, Jack Heinemann, Hartmut Meyer, Vandana Shiva and Brian Wynne, 2015. No scientific consensus on GMO safety. *Environmental Sciences Europe* 2015, 27:4. Confere posfácio.

poderiam contradizer o modelo agroindustrial dominante, aqueles autores construíram os conhecimentos aqui divulgados, evidenciando divergências e reduzindo a obscuridade imposta por interesses de poucos.

Em paralelo, vale dedicar este trabalho a todos os cientistas, formadores de opinião e atores políticos que contribuem significativamente para o avanço da ciência e a sua democratização e levam o debate para quem é diretamente envolvido pelas questões de biossegurança relativas ao uso de plantas transgênicas em escala comercial: os agricultores e os consumidores.

Enfim, deve-se reconhecer e homenagear a existência de cientistas e pesquisadores sérios e comprometidos com a proteção da saúde pública, a preservação do meio ambiente e o reconhecimento do papel sociocultural da agricultura que ocupam cargos nos órgãos reguladores de biossegurança e que pautam sua atuação pelo Princípio da Precaução. Esses cidadãos constituem uma minoria nesses espaços de confrontação, onde o conhecimento científico tende a ser minimizado por interesses político-econômicos de determinados setores da sociedade. Frequentemente sofrem assédio moral, são profissionalmente desqualificados ou por vezes, simplesmente ignorados. Apesar disso, alguns se mantêm naqueles espaços, conscientes da sua responsabilidade social. Esse trabalho deveria servir-lhes de conforto, ao ressaltar que, apesar de manterem vozes discordantes compondo o minguado conjunto de votos vencidos naqueles espaços de decisão, seus posicionamentos são respaldados por pelo menos 750 estudos publicados na literatura científica especializada.

Posfácio

As discussões referentes à segurança dos produtos transgênicos recrudesceram em 2015, com algumas publicações de grande repercussão internacional. Nos três primeiro meses do ano – depois de concluída a pesquisa que deu base a este livro – foram sistematizados 19 artigos (uma média equivalente a cerca de seis por mês!), disponibilizados na bibliografia internacional, apontando preocupações quanto a novos aspectos de riscos das plantas transgênicas, o que justifica sua inclusão neste posfácio, classificados por ordem alfabética. Como afirmado anteriormente, a sistematização de estudos científicos que apontam para riscos e incertezas das plantas transgênicas é uma tarefa que se encontra ainda em seus primórdios. De fato, a polêmica científica a seu respeito continua intensa e atual, e sua permanência ao longo dos 25 últimos anos deveria ser interpretada como alerta para a necessidade de se adotar uma perspectiva precaucionária nas tomadas de decisão.

Cerca da metade desses artigos apontam riscos para a saúde humana e animal e para o meio ambiente associados ao uso de glifosato. Entre estes merece destaque o estudo publicado pela Agência Internacional de Pesquisa sobre o Câncer[73] (IARC, sigla em inglês), vinculada a Organização Mundial da Saúde (OMS), que classifica o glifosato como "possível cancerígeno", que é aúltima etapa antes da classificação como "cancerígeno confirmado". Apesar de as opiniões daquele órgão não possuírem caráter vinculante com as políticas dos países membros, a Anvisa afirmou recentemente que o produto será reavaliado no Brasil. Com isso, é a pedra angular do setor das biotecnologias que está sendo visada.

Além disso, em que pese a correlação evidente entre os riscos do glifosato para a saúde e aqueles associados ao consumo de plantas trans-

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[73] O estudo chancelado pela IARC serviu de base para um alerta sobre impactos do uso de agrotóxicos no Brasil, divulgado no mês seguinte pelo Instituto Nacional do Câncer (Inca). Disponível em: http://www1.inca.gov.br/inca/Arquivos/comunicacao/posicionamento_do_inca_ sobre_os_agrotoxicos_06_abr_15.pdf.

gênicas tolerantes ao herbicida (que contém altos resíduos de glifosato), quatro outros estudos apontam diretamente para riscos para a saúde associados ao consumo de plantas transgênicas Bt e/ou TH.

Outro artigo que merece destaque nesse posfácio é apresentado por Crisp et al. (2015). Analisando e comparando o genoma de dezenas de espécies do mundo animal (moscas, nematoides e vertebrados, inclusive primatas), os autores observam que a transferência horizontal de genes entre espécies ao longo da evolução resultou na aquisição de dezenas ou centenas de genes "exógenos", hoje amplamente envolvidos no metabolismo dos organismos receptores. No caso do ser humano, a pesquisa identificou pelo menos 33 exemplos de genes adquiridos por meio de THG. Esses resultados desafiam o obscurantismo imposto por órgãos reguladores que, como a CTNBio, se recusam sistematicamente a avaliar riscos associados à THG entre plantas transgênicas e organismos do ambiente. A recusa se dá sob pretexto de que a probabilidade de THG vir a ocorrer seria nula, muito remota e/ou cientificamente não comprovada.

Por outro lado, no que diz respeito à eficiência da tecnologia e suas falhas, dois novos artigos mostram fragilidades teóricas nos pressupostos que sustentam o uso das biotecnologias agrícolas do tipo Bt (que sintetizam toxinas inseticidas) e TH (que visam "limpar" as lavouras com banhos de herbicidas). De fato, Jaludin et al. (2015) observaram populações de plantas ruderais (*Eleusine indica*) que evoluíram para adquirir um perfil genético/metabolômico insensível simultaneamente aos herbicidas a base de glifosato, glufosinato de amônio, paraquat e herbicidas inibidores de ACCase. A presença em campo de tais populações é um sinal claro do fracasso previsível da tecnologia. A comercialização de plantas transgênicas tolerantes a vários herbicidas não resolverá os problemas de manejo já existentes, associados às populações de plantas ruderais resistentes a herbicidas. Em paralelo, Carrière et al. (2015) reforçam a ineficiência relativa da piramidação de transgenes inseticidas em plantas Bt. De fato, populações de insetos adquirem perfil de resistência cruzada contra vários tipos de proteínas Bt, mesmo quando estas possuem mecanismos de ação molecular distintos.

Enfim, ainda sobre questões relativas ao uso comercial de plantas transgênicas, dois outros artigos reforçam evidências de falhas da tecnologia. Revelam que alguns transgenes não conseguem se expressar corretamente (de um ponto de vista quantitativo) e impor novas funções aos organismos modificados, sem alterar outras de suas características. Em planta de café, os pesquisadores observaram correlação negativa entre a síntese de Cry1Ac e a velocidade de crescimento da planta. Em outro estudo,que focalizou o milho Mon810, os autores observaram modificação do perfil de expressão das proteínas Bt sob pressão de condições de estresse ambiental, resultando em alterações significativas da quantidade de toxina realmente produzida na variedade testada. Ora, cabe lembrar que flutuações na quantidade de proteínas Cry sintetizadas representam um fator de risco para o desenvolvimento/fortalecimento de populações de insetos resistentes ao Bt, impactando negativamente o sucesso da tecnologia em sua pretensão de controlar “insetos-praga”.

Andreassen, M.; Rocca, E.; Bøhn, T.; Wikmark, O-G.; Berg, J.; Løvik, M.; Traavik, T.; Nygaard, U. 2015. Humoral and cellular immune responses in mice after airway administration of *Bacillus thuringiensis* Cry1Ab and MON810 cry1Ab-transgenic maize. *Food and Agricultural Immunology*, 26:4, 521-537, DOI:10.1080/09540105.2014.988128.

Genetically modified (GM) crops may bring new proteins with immunogenic and allergenic properties into the food and feed chains. The most commonly grown GM maize, MON810, expresses a modified version of the insecticidal Cry1Ab protein originating in the soil bacterium*Bacillus thuringiensis* (Bt). Immune reactions following inhalation of pollen and debris from such plants have been scarcely studied. We exposed BALB/c mice to purified Cry1Ab proteins and Cry1Ab-containing MON810 plant materials by intranasal installation. No anti-Cry1Ab antibodies were detected following exposure to the plant materials. Exposure to purified Cry1Ab resulted in specific anti-Cry1Ab IgG1 and IgE production, indicating inherent immunogenicity and allergenicity. Mice exposed to leaf extracts from both MON810 and unmodified maize demonstrated influx of lymphocytes and eosinophils in the broncho-alveolar lavage, and increased cytokine release in mediastinal lymph node cells. The results indicate that the airway exposure to Cry1Ab proteins may be a route of practical relevance.

Artigo completo disponível em http://www.tandfonline.com/doi/pdf/10.1080/09540105.2014.988128

Carrière, Y.; Crickmore, N.; Tabashnik, B. 2015. Optimizing pyramided transgenic Bt crops for sustainable pest management. *Nature Biotechnology*, ISSN 1087-0156.

Transgenic crop pyramids producing two or more Bacillus thuringiensis (Bt) toxins that kill the same insect pest have been widely used to delay evolution of pest resistance. To assess the potential of pyramids to achieve this goal, we analyze data from 38 studies that report effects of ten Bt toxins used in transgenic crops against 15 insect pests. We find that compared with optimal low levels of insect survival, survival on currently used pyramids is often higher for both susceptible insects and insects resistant to one of the toxins in the pyramid. Furthermore, we find that cross-resistance and antagonism between toxins used in pyramids are common, and that these problems are associated with the similarity of the amino acid sequences of domains II and III of the toxins, respectively. This analysis should assist in future pyramid design and the development of sustainable resistance management strategies.

http://www.nature.com/nbt/journal/v33/n2/full/nbt.3099.html#close

Crisp, A.; Boschetti, C.; Perry, M.; Tunnacliffe, A.; Micklem, G. 2015. Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. *Genome Biology*, 16(1): 50, doi: 10.1186/s13059-015-0607-3.

Background: A fundamental concept in biology is that heritable material, DNA, is passed from parent to offspring, a process called vertical gene transfer. An alternative mechanism of gene acquisition is through horizontal gene transfer (HGT), which involves movement of genetic material between different species. HGT is well-known in single-celled organisms such as bacteria, but its existence in higher organisms, including animals, is less well established, and is controversial in humans.
Results: We have taken advantage of the recent availability of a sufficient number of high-quality genomes and associated transcriptomes to carry out a detailed examination of HGT in 26 animal species (10 primates, 12 flies and four nematodes) and a simplified analysis in a further 14 vertebrates. Genome-wide comparative and phylogenetic analyses show that HGT in animals typically gives rise to tens or hundreds of active 'foreign' genes, largely concerned with metabolism. Our analyses suggest that while fruit flies and nematodes have continued to acquire foreign genes throughout their evolution, humans and other primates have gained relatively few since their common ancestor. We also resolve the controversy surrounding previous evidence of HGT in humans and provide at least 33 new examples of horizontally acquired genes.
Conclusions: We argue that HGT has occurred, and continues to occur, on a previously unsuspected scale in metazoans and is likely to have contributed to biochemical diversification during animal evolution.

Artigo completo disponível em http://genomebiology.com/2015/16/1/50

Cuhra, M.; Traavik, T.; Dando, M.; Primicerio, R.; Holderbaum, D.; Bøhn, T. 2015. Glyphosate-residues in Roundup-Ready soybean impair *Daphnia magna* life-cycle. *Journal of Agricultural Chemistry and Environment*, 4, 24-36. doi: 10.4236/jacen.2015.41003.

Herbicide tolerant plants such as Roundup-Ready soybean contain residues of glyphosate herbicide. These residues are considered safe and previous animal-feeding-studies have failed to find negative effects related to such chemical residues. The present study tests 8 experimental soy- meal diets as feed in groups (each containing 20 individuals) of test-animals (D. magna). The diets have different levels of glyphosate residues and we show that animal growth, reproductive maturity and number of offspring are correlated with these chemicals. The tested soybeans are from ordinary agriculture in Iowa USA and the residues are below the regulatory limits. Despite this, clear negative effects are seen in life-long feeding. The work enhances the need for including analysis of herbicide residues in future assessment of GMO.

Artigo completo disponível em http://www.scirp.org/journal/PaperInformation.aspx?paperID=53681#.VRGnlvldWt8

Guyton, K.; Loomis, D.; Grosse, Y.; El Ghissassi, F.; Benbrahim-Tallaa, L.; Guha, N.; Scoccianti, C.; Mattock, H.; Straif, K.; International Agency for Research on Cancer Monograph Working Group - IARC (Lyon, France). 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *The Lancet Oncology*, pii: S1470-2045(15)70134-8. doi: 10.1016/S1470-2045(15)70134-8.

In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be published as volume 112 of the IARC Monographs.

http://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(15)70134-8/fulltext

REV - Hilbeck, A.; Binimelis, R.; Defarge, N.; Steinbrecher, R.; Székács, A.; Wickson, F.; Antoniou, M.; Bereano, P.; Clark, E.; Hansen, M.; Novotny, E.; Heinemann, J.; Meyer, H.; Shiva, V.; Wynne, B. 2015. No scientific consensus on GMO safety. *Environmental Sciences Europe*, 27:4.

A broad community of independent scientific researchers and scholars challenges recent claims of a consensus over the safety of genetically modified organisms (GMOs). In the following joint statement, the claimed consensus is shown to be an artificial construct that has been falsely perpetuated through diverse fora. Irrespective of contradictory evidence in the refereed literature, as documented below, the claim that there is now a consensus on the safety of GMOs continues to be widely and often uncritically aired. For decades, the safety of GMOs has been a hotly controversial topic that has been much debated around the world. Published results are contradictory, in part due to the range of different research methods employed, an inadequacy of available procedures, and differences in the analysis and interpretation of data. Such a lack of consensus on safety is also evidenced by the agreement of policymakers from over 160 countries - in the UN?s Cartagena Biosafety Protocol and the Guidelines of the Codex Alimentarius - to authorize careful case-by-case assessment of each GMO by national authorities to determine whether the particular construct satisfies the national criteria for `safe?. Rigorous assessment of GMO safety has been hampered by the lack of funding independent of proprietary interests. Research for the public good has been further constrained by property rights issues, and by denial of access to research material for researchers unwilling to sign contractual agreements with the developers, which confer unacceptable control over publication to the proprietary interests.The joint statement developed and signed by over 300 independent researchers, and reproduced and published below, does not assert that GMOs are unsafe or safe. Rather, the statement concludes that the scarcity and contradictory nature of the scientific evidence published to date prevents conclusive claims of safety, or of lack of safety, of GMOs. Claims of consensus on the safety of GMOs are not supported by an objective analysis of the refereed literature.

Artigo completo disponível em http://www.enveurope.com/content/27/1/4/abstract

Jalaludin, A.; Yu, Q.; Powles, S. 2015. Multiple resistance across glufosinate, glyphosate, paraquat and ACCase-inhibiting herbicides in an *Eleusine indica* population. *Weed Research*, 55, 82–8.

An *Eleusine indica* population was previously reported as the first global case of field-evolved glufosinate resistance. This study re-examines glufosinate resistance and investigates multiple resistance to other herbicides in the population. Dose–response experiments with glufosinate showed that the resistant population is 5-fold and 14-fold resistant relative to the susceptible population, based on $GR_{50}$and $LD_{50\ R}/S$ ratio respectively. The selected glufosinate-resistant subpopulation also displayed a high-level resistance to glyphosate, with the respective GR_{50} and $LD_{50\ R}/S$ ratios being 12- and 144-fold. In addition, the subpopulation also displayed a level of resistance to paraquat and ACCase-inhibiting herbicides fluazifop-P-butyl, haloxyfop-P-methyl and butroxydim. ACCase gene sequencing revealed that the Trp-2027-Cys mutation is likely responsible for resistance to the ACCase inhibitors examined. Here, we confirm glufosinate resistance and importantly, we find very high-level glyphosate resistance, as well as resistance to paraquat and ACCase-inhibiting herbicides. This is the first confirmed report of a weed species that evolved multiple resistance across all the three non-selective global herbicides, glufosinate, glyphosate and paraquat.

Artigo completo disponível em http://onlinelibrary.wiley.com/doi/10.1111/wre.12118/full

Jayasumana, C.; Paranagama, P.; Agampodi, S.; Wijewardane, C.; Gunatilake, S.; Siribaddana, S. 2015. Drinking well water and occupational exposure to herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka. *Environmental Health*, 14(1):6.

Background: The chronic kidney disease of unknown etiology (CKDu) among paddy farmers in was first reported in 1994 and has now become most important public health issue in dry zone of Sri Lanka. The objective was to identify risk factors associated with the epidemic in an area with high prevalence.
Methods: A case control study was carried out in Padavi-Sripura hospital in Trincomalee district. CKDu patients were defined using health ministry criteria. All confirmed cases (N = 125) fulfilling the entry criteria were recruited to the study. Control selection (N = 180) was done from people visiting the hospital for CKDu screening. Socio-demographic and data related to usage of applying pesticides and fertilizers were studied. Drinking water was also analyzed using ICP-MS and ELISA to determine the levels of metals and glyphosate.
Results: Majority of patients were farmers (N = 107, 85.6%) and were educated up to 'Ordinary Level' (N = 92, 73.6%). We specifically analyzed for the effect modification of, farming by sex, which showed a significantly higher risk for male farmers with OR 4.69 (95% CI 1.06-20.69) in comparison to their female counterparts. In the multivariable analysis the highest risk for CKDu was observed among participants who drank well water (OR 2.52, 95% CI 1.12-5.70) and had history of drinking water from an abandoned well (OR 5.43, 95% CI 2.88-10.26) and spray glyphosate (OR 5.12, 95% CI 2.33-11.26) as a pesticide. Water analysis showed significantly higher amount of hardness, electrical conductivity and glyphosate levels in abandoned wells. In addition Ca, Mg, Ba, Sr, Fe, Ti, V and Sr were high in abandoned wells. Surface water from reservoirs in the endemic area also showed contamination with glyphosate but at a much lower level. Glyphosate was not seen in water samples in the Colombo district.
Conclusion: The current study strongly favors the hypothesis that CKDu epidemic among farmers in dry zone of Sri Lanka is associated with, history of drinking water from a well that was abandoned. In addition, it is associated with spraying glyphosate and other pesticides in paddy fields. Farmers do not use personnel protective equipments and wears scanty clothing due to heat when spraying pesticides.

Artigo completo disponível em http://www.ehjournal.net/content/pdf/1476-069X-14-6.pdf

COM - Kloor, K. 2015. Agricultural researchers rattled by demands for documents from group opposed to GM foods. *Science*, Vol 347, ISSUE 6223, p.699.

Sem resumo.

Artigo completo disponível em http://news.sciencemag.org/scientific-community/2015/02/agricultural-researchers-rattled-demands-documents-group-opposed-gm

Kurenbach, B.; Marjoshi, D.; Amábile-Cuevas, C.; Ferguson, G.; Godsoe, W.; Gibson, P.; Heinemann, J. 2015. Sublethal exposure to commercial formulations of the herbicides dicamba, 2,4-dichlorophenoxyacetic acid, and glyphosate cause changes in antibiotic susceptibility in *Escherichia coli* and Salmonella enterica serovar Typhimurium. *mBio*, 6(2):e00009-15. Doi:10.1128/mBio.00009-15.

Biocides, such as herbicides, are routinely tested for toxicity but not for sublethal effects on microbes. Many biocides are known to induce an adaptive multiple-antibiotic resistance phenotype. This can be due to either an increase in the expression of efflux pumps, a reduced synthesis of outer membrane porins, or both. Exposures of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to commercial formulations of three herbicides—dicamba (Kamba), 2,4-dichlorophenoxyacetic acid (2,4-D), and glyphosate (Roundup)—were found to induce a changed response to antibiotics. Killing curves in the presence and absence of sublethal herbicide concentrations showed that the directions and the magnitudes of responses varied by herbicide, antibiotic, and species. When induced, MICs of antibiotics of five different classes changed up to 6-fold. In some cases the MIC increased, and in others it decreased. Herbicide concentrations needed to invoke the maximal response were above current food maximum residue levels but within application levels for all herbicides. Compounds that could cause induction had additive effects in combination. The role of *soxS*, an inducer of the AcrAB efflux pump, was tested in β-galactosidase assays with *soxS-lacZ* fusion strains of *E. coli*. Dicamba was a moderate inducer of the *sox* regulon. Growth assays with Phe-Arg β-naphtylamide (PAβN), an efflux pump inhibitor, confirmed a significant role of efflux in the increased tolerance of*E. coli* to chloramphenicol in the presence of dicamba and to kanamycin in the presence of glyphosate. Pathways of exposure with relevance to the health of humans, domestic animals, and critical insects are discussed.

Artigo completo disponível em http://mbio.asm.org/content/6/2/e00009-15.full

Moore, H.; Chivers, D.; Ferrari, M. 2015. Sub-lethal effects of Roundup™ on tadpole anti-predator responses. *Ecotoxicology and Environmental Safety*, 111, 281-285.

Roundup™ is a commonly used pesticide applied to agriculture and forest habitats. These areas are generally ideal for amphibians due to the presence of small, ephemeral water bodies. While Roundup™ has been shown to have lethal effects on many species of amphibians, effects on behaviour and sensory perception have yet to be considered. Here, we exposed wood frog tadpoles to a sub-lethal concentration of Roundup™ and showed that the ability of tadpoles to respond to injured conspecific cues, an important source of information regarding local predation risk, was impaired. Subsequent experiments revealed that impaired responses likely result from a chemical reaction between the Roundup™ and the cues and that tadpoles chronically exposed to Roundup™ had reduced basal movement rates compared with unexposed tadpoles. Our data demonstrate that environmentally-relevant concentrations of Roundup™ can drastically alter movement and anti-predator responses of tadpoles, with potential negative consequences for the population.

http://www.sciencedirect.com/science/article/pii/S0147651314004801

Oraby, H.; Kandil, M.; Shaffie, N.; Ghaly, I. 2015. Biological impact of feeding rats with a genetically modified-based diet. *Turkish Journal of Biology*, 39.

This work was conducted in the context of postmarketing biosafety assessment of genetically modified products. It presents a systematic approach based on a chronic toxicity study on Wistar albino rats, with a range of combined parameters including biochemical, histopathological, and cytogenetic to evaluate the negative impact of a genetically modified (GM) diet on animal health. Histopathological and biochemical analysis procedures were performed in the liver, kidney, and testis. Cytogenetic analysis was evaluated in germ cells and the liver. The results revealed that the laboratory diet used in our investigation was proved experimentally, using the PCR assay, to contain genetically modified components without being labeled as such. The results of all parameters evaluated in our investigation were consistent and confirm that the GM diet fed to rats for 30, 60, or 90 days has deleterious histopathological and histochemical impacts. Biochemical alterations in alanine aminotransferase, aspartate aminotransferase, creatinine, uric acid, and malondialdehyde concentrations were also observed. Genotoxicity of the GM diet was also demonstrated in germ cells as increased numbers of cells with chromosomal aberrations and in liver cells as increased ratios of DNA fragmentation. In conclusion, the results of the present work indicate that there are health hazards linked to the ingestion of diets containing genetically modified components.

Artigo completo disponível em http://journals.tubitak.gov.tr/biology/issues/biy-15-39-2/biy-39-2-11-1406-61.pdf

Perthuis, B.; Vassal, J-M.; Fenouillet, C.; Leroy, T. 2015. Cry1Ac insecticidal protein levels in genetically modified *Coffea canephora* Pierre coffee plants were negatively correlated with the growth speed in a field experiment. *Euphytica*, Volume 202, Issue 3, pp 373-383.

Genetically-modified coffee clones (GMCs) were presented in a previous work. They were created from a single commercial clone of *Coffea canephora* Pierre (clone 126). Therefore they all have the same genotype, except for the localization of the transgenic insertions. They synthesize the *Bacillus thuringiensis* endotoxin Cry1Ac against *Leucoptera coffeella* Guérin-Méneville, a secondary pest of*C. canephora* and one of the main pest of *C. arabica* in South America. The synthetic *Cry1Ac* gene is regulated by the *EF1*α*-A1* promoter of *Arabidopsis thaliana* (L.) Heynh. They were tested in an experimental field for their resistance against the pest insect in a previous work. In the present work, levels of Cry1Ac were measured in the leaves. The insecticidal protein was evenly distributed in all the leaves. Cry1Ac levels were measured once in the coffee plants of a sensitive GMC and of 14 resistant ones grown in the experimental field and in plants grown in a greenhouse. Some resistant GMCs contained higher levels but it is not possible to confirm that it would be enough for a sustainable resistance to the pest. Growth speed was variable in the plot. The correlation with plant height and other indicators of plant growth was examined. Cry1Ac levels in the GMCs were negatively correlated with growth speed. The latter was not statistically influenced neither by Cry1Ac synthesis nor by the genetic modification in itself as seen by comparing the GMCs and the unmodified control clone 126. Hence the conclusion is that the growth conferred to field-grown plants by environmental factors and especially the soil was probably the underlying cause of the negative correlation. Other field experiments would be necessary in order to confirm this result. It would be important that the genetic construct inserted in these GMCs and mainly the *EF1*α*-A1* promoter of the *Cry1Ac* gene be reconsidered since Cry1Ac levels might be too low to provide efficient and sustainable protection against *L. coffeella* in a highly favourable environment for coffee plants. Alternatives are discussed.

http://link.springer.com/article/10.1007%2Fs10681-014-1258-2

Reisig, D.; Reay-Jones, F. 2015. Inhibition of *Helicoverpa zea* (*Lepidoptera*: *Noctuidae*) growth by transgenic corn expressing Bt toxins and development of resistance to Cry1Ab. *Environmental Entomology*, 1–11; DOI: 10.1093/ee/nvv076.

Transgenic corn, Zea mays L., that expresses the Bacillus thuringiensis (Bt) toxin Cry1Ab is only moderately toxic to Helicoverpa zea (Boddie) and has been planted commercially since 1996. Growth and development of H. zea was monitored to determine potential changes in susceptibility to this toxin over time. Small plots of corn hybrids expressing Cry1F, Cry1FCry1Ab, Cry1AbCry3Bb1, Cry1A.105Cry2Ab2Cry3Bb1, Cry1A.105Cry2Ab2, and Vip3Aa20Cry1AbmCry3A were planted in both 2012 and 2013 in North and South Carolina with paired non-Bt hybrids from the same genetic background. H. zea larvae were sampled on three time periods from ears and the following factors were measured: kernel area injured (cm2) by H. zea larvae, larval number per ear, larval weight, larval length, and larval head width. Pupae were sampled on a single time period and the following factors recorded: number per ear, weight, time to eclosion [the emergence of an insect from the pupa case], and the number that eclosed. There was no reduction in larval weight, number of insect entering the pupal stadium, pupal weight, time to eclosion, and number of pupae able to successfully eclose to adulthood in the hybrid expressing Cry1Ab compared with a non-Bt paired hybrid. As Cry1Ab affected these in 1996, H. zea may be developing resistance to Cry1Ab in corn, although these results are not comprehensive, given the limited sampling period, size, and geography. We also found that the negative impacts on larval growth and development were greater in corn hybrids with pyramided traits compared with single traits.

http://ee.oxfordjournals.org/content/early/2015/05/20/ee.nvv076

Rótolo, G.; Francis, C.; Craviotto, R.; Ulgiati, S. 2015. Environmental assessment of maize production alternatives: Traditional, intensive and GMO-based cropping patterns. *Ecological Indicators***,** Volume 57, Pages 48–60.

The evolution of maize production patterns in Argentina is evaluated over the last 25 years to compare costs, benefits, environmental performance and sustainability as well as to identify the main driving sources and improvement potential. Results from Argentina cropping systems are compared to other systems worldwide in order to put the Argentina results in a broader context. The study focuses on three farming categories: (1) traditional, low-intensity systems, (2) conventional, high-intensity systems, and (3) GMO-based cropping systems. Low input intensity systems include traditional cropping patterns with seed selection by farmers and conventional hybrid seed coupled to plowing and crop-animal rotation techniques; high input intensity systems use conventional hybrid seeds and recommended chemicals, irrigation and machinery with important soil erosion consequences; and GMO-based cropping systems use herbicide resistant transgenic hybrids, pesticides, higher fertilizer rates, and no-till practices. In each of the three cases, input flows are compared to the achieved yield (in mass and income terms) to better understand relative efficiencies and options for improvement. The study of GMO systems required a preliminary investigation of GMO seed production by seed companies, where a large investment in terms of prior knowledge and high-tech laboratory research is required. The assessments used the Emergy Accounting (EMA) approach. EMA includes material, energy, labor, money, and knowledge flows into the assessment and expands its focus over larger time and spatial scales than conventional economic and cumulative energy demand methods. Emergy-based environmental indicators of grain production for high-intensity hybrid and GMO systems both show a lower performance than low-intensity, traditional patterns in terms of resource return, renewability and sustainability. The fraction of renewability in low-intensity systems is between 28% and 63%, while it is between 8% and 26% for high-intensity hybrid and GMO systems. Calculated indicators also show that GMO-based maize production patterns do not guarantee the expected improvement over conventional high-intensity cropping systems or low-intensity systems in terms of performance and sustainability. Strong reliance on nonrenewable resources and technology, as well as role of direct and indirect labor costs are important factors in determining long-term sustainability and environmental stability of maize production systems.

http://www.sciencedirect.com/science/article/pii/S1470160X15001661

Seneff, S.; Swanson, N.; Li, C. 2015. Aluminum and glyphosate can synergistically induce pineal gland pathology: Connection to gut dysbiosis and neurological disease. *Agricultural Sciences*, 6, 42-70.

Many neurological diseases, including autism, depression, dementia, anxiety disorder and Parkinson's disease, are associated with abnormal sleep patterns, which are directly linked to pineal gland dysfunction. The pineal gland is highly susceptible to environmental toxicants. Two pervasive substances in modern industrialized nations are aluminum and glyphosate, the active ingredient in the herbicide, Roundup?. In this paper, we show how these two toxicants work synergistically to induce neurological damage. Glyphosate disrupts gut bacteria, leading to an overgrowth of Clostridium difficile. Its toxic product, p-cresol, is linked to autism in both human and mouse models. p-Cresol enhances uptake of aluminum via transferrin. Anemia, a result of both aluminum disruption of heme and impaired heme synthesis by glyphosate, leads to hypoxia, which induces increased pineal gland transferrin synthesis. Premature birth is associated with hypoxic stress and with substantial increased risk to the subsequent development of autism, linking hypoxia to autism. Glyphosate chelates aluminum, allowing ingested aluminum to bypass the gut barrier. This leads to anemia-induced hypoxia, promoting neurotoxicity and damaging the pineal gland. Both glyphosate and aluminum disrupt cytochrome P450 enzymes, which are involved in melatonin metabolism. Furthermore, melatonin is derived from tryptophan, whose synthesis in plants and microbes is blocked by glyphosate. We also demonstrate a plausible role for vitamin D3 dysbiosis in impaired gut function and impaired serotonin synthesis. This paper proposes that impaired sulfate supply to the brain mediates the damage induced by the synergistic action of aluminum and glyphosate on the pineal gland and related midbrain nuclei.

Artigo completo disponível em http://www.scirp.org/journal/PaperInformation.aspx?paperID=53106#.VOR1ufldWt8

Lima, I.; Baumeier, N.; Rosa, R.; Campelo, P.; Rosa, E. 2015. Influence of glyphosate in planktonic and biofilm growth of *Pseudomonas aeruginosa. Brazilian Journal of Microbiology*, 45(3): 971–975.

This study evaluated the impact of different concentrations of glyphosate (Rondup®) on planktonic and biofilm growth of *P. aeruginosa*. Aerobic and anaerobic cultures of *P. aeruginosa* ATCC®15442 inoculated in MHB + glyphosate (0.845 ppm, 1.690 ppm, 8.45 ppm, 16.90 ppm, 84.50 ppm, 169 ppm, 845 ppm, and 1690 ppm) and cultured in normoxia and anoxia, following their OD_{560nm} every hour for 24 h. Biofilms of adapted cells were formed in the presence of glyphosate (0.845 to 1690 ppm) in normoxia and anoxia for 36 h. Glyphosate at concentrations higher than 84.5 ppm reduces the cell density of planktonic aerobic cultures ($p < 0.05$). However, these same concentrations favor the planktonic anaerobic growth ($p < 0.05$). On the other hand, the herbicide favors a slight growth of biofilms in a concentration-dependent manner up to 84.5 ppm ($p > 0.05$), and more pronounced over 169 ppm. Anaerobic biofilms have their growth more readily favored ($p < 0.05$), regardless of concentration. In a concentration-dependent manner, glyphosate interferes with the growth ability of *P. aeruginosa* ATCC®15442.

Artigo completo disponível em http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4204984/

Webster, T.; Santos, E. 2015. Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup. *BMC Genomics*, 16.

Background: Glyphosate, the active ingredient in Roundup formulations, is the most widely used herbicide worldwide, and as a result contaminates surface waters and has been detected in food residues, drinking water and human urine, raising concerns for potential environmental and human health impacts. Research has shown that glyphosate and Roundup can induce a broad range of biological effects in exposed organisms, particularly via generation of oxidative stress. However, there has been no comprehensive investigation of the global molecular mechanisms of toxicity of glyphosate and Roundup for any species. We aimed to characterise and compare the global mechanisms of toxicity of glyphosate and Roundup in the liver of brown trout (*Salmo trutta*), an ecologically and economically important vertebrate species, using RNA-seq on an Illumina HiSeq

2500 platform. To do this, we exposed juvenile female brown trout to 0, 0.01, 0.5 and 10 mg/L of glyphosate and Roundup (glyphosate acid equivalent) for 14 days, and sequenced 6 replicate liver samples from each treatment.

Results: We assembled the brown trout transcriptome using an optimised *de novo* approach, and subsequent differential expression analysis identified a total of 1020 differentially-regulated transcripts across all treatments. These included transcripts encoding components of the antioxidant system, a number of stress-response proteins and pro-apoptotic signalling molecules. Functional analysis also revealed over-representation of pathways involved in regulating of cell-proliferation and turnover, and up-regulation of energy metabolism and other metabolic processes.

Conclusions: These transcriptional changes are consistent with generation of oxidative stress and the widespread induction of compensatory cellular stress response pathways. The mechanisms of toxicity identified were similar across both glyphosate and Roundup treatments, including for environmentally relevant concentrations. The significant alterations in transcript expression observed at the lowest concentrations tested raises concerns for the potential toxicity of this herbicide to fish populations inhabiting contaminated rivers.

http://www.biomedcentral.com/1471-2164/16/32

Trtikova, M.; Wikmark, O.; Zemp, N.; Widmer, A.; Hilbeck, A. 2015. Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. *PLOS ONE*, 10(4): e0123011. doi:10.1371/journal.pone.0123011.

Bt protein content in transgenic insect resistant (Bt) maize may vary between tissues within plants and between plants growing under different environmental conditions. However, it is unknown whether and how Bt protein content correlates with transgene expression, and whether this relationship is influenced by stressful environmental conditions. Two Bt maize varieties containing the same transgene cassette (MON 810) were grown under optimal and stressful conditions. Before and during stress exposure, the upper leaves were analysed for transgene expression using quantitative RT-PCR and for Bt content using ELISA. Under optimal conditions there was no significant difference in the transgene expression between the two investigated Bt maize varieties whereas Bt protein content differed significantly. Transgene expression was correlated with Bt protein content in only one of the varieties. Under stressful environmental conditions we found similar transgene expressions as under optimal conditions but Bt content responded differently. These results suggest that Bt content is not only controlled by the transgene expression but is also dependent on the genetic background of the maize variety. Under stressful conditions the concentration of Bt protein is even more difficult to predict.

Artigo completo disponível em http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123011

Tudisco, R.; Calabrò, S.; Cutrignelli, M.; Moniello, G.; Grossi, M.; Mastellone, V.; Lombardi, P.; Pero, M.; Infascelli, F. 2015. Genetically modified soybean in a goat diet: Influence on kid performance. *Small Ruminant Research*, Pages 67–74.

The in vivo and *post mortem* performance and serum immunoglobulin G (IgG) concentration in kids born from goats fed conventional (group C) or genetically modified (group T) soybean meal were evaluated. The goat colostrum quality, in terms of chemical composition, as well as immunoglobulin concentration, and the presence of feed DNA fragments were also investigated. Kid birth weights were similar, while significantly ($P < 0.05$) higher in those born from goats in group C at day 30 and at slaughtering. In addition, kids from mothers fed conventional soybean recorded significant ($P < 0.05$) higher height at the withers and chest width. Concerning the post mortem measurements, only carcass weights were significantly affected by the treatment resulting in lighter T kids ($P < 0.05$). Colostrum from the treated groups recorded a significantly ($P < 0.01$)

lower percentage of protein and fat. Similarly, both chemical parameters significantly differed in milk collected 15 days after kidding, although these differences disappeared in the successive samplings. Both colostrum and kids serum IgG concentration were significantly ($P < 0.01$) lower in the treated groups. Transgenic target DNA sequences (35S and CP4 EPSPS) were not detected in colostrum from goats that received a diet containing conventional soybean meal. By contrast, transgenic DNA fragments were amplified ($P < 0.05$) in samples from goats that received the transgenic soybean

http://www.sciencedirect.com/science/article/pii/S0921448815000528

Young, F.; Ho, D.; Glynn, D.; Edwards, V. 2015. Endocrine disruption and cytotoxicity of glyphosate and roundup in human JAr cells *in vitro*. *Integrative Pharmacology, Toxicology and Genotoxicology*; doi: 10.15761/IPTG.1000104, Volume 1(1): 12-19.

The toxicity of the active molecule in herbicides has been used to determine safe concentrations, because other components are considered inert. Roundup, which contains the active molecule Glyphosate, was described as an endocrine disrupter because non-cytotoxic concentrations inhibited progesterone synthesis in vitro. Human chorioplacental JAr cells synthesise progesterone, and increase synthesis when stimulated by chorionic gonadotrophin (hCG), or the transduction molecule cAMP. JAr cells were exposed to two Roundup formulations, and compared with the same concentrations of glyphosate ± cAMP, or ± hCG for 1, 4, 24, 48 or 72h. The surviving viable cells were quantified using an MTT assay, and progesterone was measured in an ELISA. hCG and cAMP stimulated progesterone synthesis by cells in vitro as expected. In contrast to previous reports, JAr cell death preceded decreased progesterone synthesis, and steroidogenesis was unaffected by low, non-cytotoxic concentrations of Roundup or glyphosate. Roundup was more cytotoxic than glyphosate alone; the 24h EC50 was 16mM for glyphosate, but 0.008mM when glyphosate was in a 7.2g/L Roundup formulation. Significant cytotoxicity was caused by glyphosate in Roundup ($p<0.01$) after 24h, and cytotoxicity was observed in vitro after exposure toa range of concentrations comparable to the Australian Drinking Water Guidelines.Endocrine disruption effects were secondary to cytotoxicity. Roundup was more cytotoxic than the same concentration of glyphosate alone, indicating that the other constituents of the herbicide are not inert. There is a compelling need to conduct in vivo studies to characterise the toxicity of glyphosate in a Roundup formulation, to facilitate re-evaluation of existing public health guidelines.

Artigo completo disponível em http://www.gmoevidence.com/wp-content/uploads/2015/03/IPTG-1-104.pdf

Glossário

Alelo (de um gene): membro de uma parte (ou série) das diferentes formas de um gene. O genótipo de um indivíduo para esse gene é o conjunto de alelos que possui. Como exemplo, podemos mencionar o caso do gene que codifica a cor das flores de muitas espécies. Um único gene controla a cor das pétalas, mas podem existir várias formas (alelos) diferentes desse gene. Uma forma pode dar a cor vermelha às pétalas, enquanto outra dará a cor branca. Assim, a cor da flor vai depender de qual desses dois alelos o organismo possui para esse gene e de como eles interagem.

Conjugação: transferência de material genético entre bactérias por contato direto entre células.

Deriva genética (ou deriva alélica): mecanismo que, atuando em consonância com a seleção natural, modifica as características das espécies ao longo do tempo. É um processo estocástico, que atua sobre as populações, modificando a frequência dos alelos e a predominância de certas características na população. A deriva genética pode conduzir ao desaparecimento de alguns alelos, especialmente em pequenas populações.

Dominante (caractere): em genética, um caractere dominante, se transmitido à progênie, será automaticamente expresso pela progênie (a progênie sintetizará a proteína Cry, por exemplo).

Exsudato: refere-se, neste caso, a um líquido fisiológico que se infiltra no solo pelas raízes.

Fenótipo (ou característica fenotípica): o fenótipo de um organismo concretiza-se por qualquer característica observável visualmente, como sua morfologia ou suas propriedades fisiológicas. O fenótipo resulta da expressão dos genes de um organismo (genótipo), bem como da influência de fatores ambientais e das possíveis interações entre si.

Fitness: os animais que deixam maior número de progênies em relação aos outros indivíduos da mesma espécie são considerados como tendo um maior *fitness*. A combinação do genótipo e do meio ambiente definem, assim, o *fitness*. Se vierem a ocorrer algumas modificações no genótipo do organismo, então o *fitness* será afetado, e a frequência desse genótipo variará nas gerações seguintes, com predominância do genótipo que apresenta o maior *fitness*.

Gene marcador de resistência aos antibióticos: as técnicas usadas para transferir um novo gene dentro do genoma de uma planta são bastante ineficientes. Poucas células realmente integram o gene de interesse (transgene Bt ou HT, por exemplo). Com o objetivo de determinar quais células integraram o transgene, alguns tipos de marcadores são necessários. Em alguns casos, genes marcadores de resistência aos antibióticos são utilizados porque são fáceis de usar. Todas as células que realmente integraram o gene marcador no seu genoma integraram ao mesmo tempo o gene de interesse. Além disso, essas células serão resistentes ao antibiótico. Basta então colocar as células transformadas em contato com o antibiótico e selecionar aquelas que sobreviverem. Mesmo que esses genes marcadores não tenham outras funções após esse processo de seleção, eles ficam como parte das plantas transgênicas e representam fatores de risco importantes.

Genótipo: o genótipo de um organismo é a sua informação genética. Todos os organismos que têm o mesmo genótipo podem não parecer ou agir da mesma forma, notadamente porque a aparência e o comportamento são também relacionados às condições ambientais e de desenvolvimento. Por outro lado, todos os organismos que apresentam uma aparência idêntica (mesmo fenótipo) não têm necessariamente o mesmo genótipo.

Grupo filogenético: em biologia, a filogenia é o estudo das relações evolutivas entre vários grupos de organismos (por exemplo, as espécies ou as populações), que são descobertas por sequenciamento molecular e matrizes de dados morfológicos. A taxonomia foi fortemente influenciada pela filogenia.

Grupo taxonômico: classificação de organismos conforme similaridades biológicas e morfológicas.

Hemocelo: nome dado a uma série de espaços (seios) entre os órgãos de insetos e alguns outros organismos. Uma mistura de sangue, linfa e líquido intersticial, chamado de hemolinfa, circula pelo hemocelo.

Introgressão: movimento de um gene de uma espécie dentro do *pool* genético de outra espécie.

Invasibilidade: uma espécie invasiva refere-se a uma espécie não nativa que afeta negativamente o hábitat onde se desenvolve em relação aos aspectos econômicos, ambientais ou ecológicos.

Leghemoglobina: proteína presente nos nódulos fixadores de nitrogênio das raízes de plantas leguminosas. É produzida pelas plantas em resposta à infecção das raízes por bactérias fixadoras de nitrogênio como parte de uma interação simbiótica entre plantas e bactérias. As raízes não infectadas por *Rhizobium* não sintetizam leghemoglobina.

Nosocomial: efeito adverso causado por um tratamento médico. Uma doença nosocomial é contraída no meio hospitalar.

Ordem: na taxonomia dos insetos, Espécies estão agrupadas em Gêneros, e estes agrupados em Famílias. A Ordem agrupa então Famílias. Ex.: Lepidópteros (borboletas), Coleópteros (besouros), Hemípteros (barbeiro)...

Origem de replicação: é uma sequência de DNA localizada num cromossomo (ou num plasmídeo), onde inicia a replicação.

Plasmídeo: molécula de DNA extracromossomial, distinta do DNA cromossomial, que é capaz de se replicar independentemente deste. Na maioria dos casos, é circular e relativamente pequena. Os plasmídios são frequentemente observados em bactérias, mas podem existir também em organismos eucariotos, como os fungos.

Pleiotropia: influência de um único gene sobre várias características fenotípicas. Consequentemente, uma mutação no gene irá afetar todas essas características simultaneamente.

Pressão de seleção: pode concretizar-se pela intensidade com que um fator ambiental tende a eliminar um organismo ou a lhe conferir vantagens adaptativas.

Procarioto: grupo de organismos que não possuem um núcleo celular (= karyon), nem qualquer organito ligado à membrana. Eles diferenciam-se dos eucariotos, que possuem um núcleo celular. A maioria dos procariontes é unicelular.

Promotor: em biologia, o promotor é uma região de DNA que facilita a transcrição de um gene em particular. Os promotores são frequentemente localizados perto dos genes que eles regulam, mas essa regra sofre numerosas exceções.

Proteólise: degradação direta (digestão) de proteínas por enzimas celulares chamadas de proteases.

Receptor: em bioquímica, um receptor é uma molécula proteica com a qual uma outra molécula pode ligar-se (peptídio, hormônio, toxina etc.). Quando a ligação ocorre (sinal), isso geralmente conduz a uma resposta celular.

Recessivo (caractere): em genética, um caractere recessivo poderá ser expresso só no caso de reprodução com um indivíduo que possui também o mesmo alelo de gene. Caso contrário, o alelo de gene poderá ser transmitido na progênie, mas não será expresso.

Retrocruzamento: cruzamento de um híbrido com um dos seus genitores (ou um indivíduo geneticamente similar ao genitor), com o objetivo de obter uma progênie com identidade genética mais próxima dos seus genitores.

Retrotransposons: elementos genéticos que podem multiplicar-se num genoma e que são ubiquitários no DNA de vários organismos eucariontes, como as plantas.

Terminador: parte de uma sequência genética que indica o fim de um gene durante a transcrição de DNA cromossômico.

Transdução: transferência de genes bacterianos entre bactérias, por meio de vírus bacterianos ou bacteriófagos.

Transformação: absorção de DNA livre do meio ambiente, sua integração no genoma hospedeiro da bactéria competente e expressão subsequente da nova informação genética adquirida.

Ubiquitários: que são presentes (ou parecem presentes) em todos os lugares no mesmo tempo; onipresentes.

Vantagem seletiva: pode concretizar-se por uma vantagem genética de um organismo sobre seus competidores, o que aumentará sua capacidade de sobrevivência e de reprodução ao longo do tempo num ambiente determinado.

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Todos os autores são integrantes do Grupo de Estudos em Agrobiodiversidade (GEA/MDA) e participaram da fundação do Movimento Ciência Cidadã (http://www.movimentocienciacidada.org/principal).

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