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In This Issue:

Information Systems for Biotechnology (ISB) provides information resources to support the environmentally responsible use of agricultural biotechnology products.

Risk Assessment News

Biotechnology and Comparative Risk Assessment

Robert K. D. Peterson

Considerable attention has been paid to potential risks and risk assessment approaches for genetically engineered (GE) organisms. In recent years, a number of regulatory agencies around the world have been formulating assessment procedures to ensure the acceptable environmental safety of GE organisms. Although there are exceptions, these procedures typically follow conventional risk assessment steps, including formulation of the problem, assessment of the effects, assessment of the exposures, and characterization of the risks.

Risk assessments for GE organisms, especially GE crops, have ranged from simple, qualitative characterizations to complex, quantitative characterizations. Regardless of the type or complexity of the assessment, nearly all risk assessments of GE crops address the risk of the crop as a standalone system, without formally and systematically considering the ecological or human-health risks posed by alternative crops and cropping systems.

Risks for crops and cropping systems posed by organic, conventional, and mutagenic approaches typically are not compared to GE crops and systems. This is interesting because many other environmental risk assessments and decisions in the U.S. are presented as part of governmentally mandated Environmental Impact Statements or Environmental Assessments. A hallmark of those assessments is the analysis of alternatives (albeit not typically formalized, comparative risk assessments) to the proposed action. Formal comparative risk analyses of alternatives would be valuable for decision-making about, and public communication of, genetic engineering technologies. Comparative risk analysis can provide a broader perspective from which to consider risks and benefits posed by genetic engineering.

In Peterson and Hulting[1] and Peterson and Shama[2], we compared multiple aspects of risk associated with different wheat production systems in the U.S. and Canada using the risk assessment paradigm.

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We chose to examine risk issues associated with wheat because wheat varieties using genetic engineering are just now emerging. Wheat varieties produced using these biotechnologies have lagged behind other crop species, but are now being developed in the case of genetic engineering and are being grown commercially in the case of mutagenic techniques. Therefore, this provided us with a unique opportunity to assess comparatively the potential environmental risks (human health, ecological, and livestock risks) associated with the different biotechnology and conventional wheat production systems.

Methods

For our assessments, we used tier 1 quantitative and qualitative risk assessment methods to compare specific environmental risks associated with genetically engineered, mutagenic, and conventional wheat production systems (specifically herbicide and protein risks) in Canada and the U.S. Risk assessment typically utilizes a tiered modeling approach extending from deterministic models (Tier 1) based on conservative assumptions to probabilistic models (Tier 4) using refined assumptions[3]. In risk assessment, conservative assumptions in lower-tier assessments represent overestimates of effect and exposure; therefore, the resulting quantitative risk values typically are conservative and err on the side of environmental safety.

Herbicide-tolerant wheat varieties have been produced using both genetically engineered (DNA recombination) and chemically induced DNA mutation techniques. Replacement of traditional herbicides with glyphosate in a glyphosate-tolerant (genetically engineered) wheat system or imazamox in an imidazolinone-tolerant (mutagenic) wheat system may alter environmental risks associated with weed management. Additionally, because both systems rely on plants that express novel proteins, the proteins and plants themselves may impose risks.

For the conventional wheat system, herbicides were considered as the only stressor in our assessment. Therefore, the conventional wheat production system served as a baseline in our analysis. For the glyphosate-tolerant wheat system, herbicides and the transgenic protein were the stressors. For the imidazolinone-tolerant wheat system, herbicides and the mutated protein were the stressors. The primary stressors then potentially affected the systems through human health

(dietary exposure for the biotech proteins and herbicides, and applicator risk for the herbicides), livestock, and ecological effects. The effects we considered in this assessment reflected primary impacts. Therefore, we presented only direct effects of the stressors on the human and ecological receptors. We did not consider economic risks or agronomic risks, such as pollen-mediated and mechanical mixing of wheat grain from different production systems, pollen-mediated gene flow to wild or weedy relatives of wheat, fallow management with herbicides, herbicide resistance in target weeds, and herbicide rotation risks to alternate crops.

Herbicide risk

The herbicide active ingredients evaluated in this study included 2,4-dichlorophenoxy acetic acid (2,4-D), bromoxynil, clodinafop, clopyralid, dicamba, fenoxaprop, flucarbazone, MCPA, metsulfuron, thifensulfuron, tralkoxydim, triallate, triasulfuron, tribenuron, and trifluralin. These active ingredients were chosen because they are used on a relatively large percentage of spring wheat acres in the U.S. and Canada. Risk associated with glyphosate and imazamox also was evaluated because of their use in glyphosate-tolerant and imidazolinone-tolerant wheat.

We characterized risks to the following ecological receptors: wild mammals, birds, nontarget terrestrial plants, nontarget aquatic plants, aquatic vertebrates, aquatic invertebrates, and groundwater. Ecological risks were assessed by integrating toxicity and exposure. To do this, risks to ecological receptors were assessed using the Risk Quotient (RQ) Method. For each ecological receptor, an RQ was calculated by dividing the Estimated Environmental Concentration (EEC) by the appropriate toxicity endpoint (e.g., the LC50).

Transgenic protein risk

Risks for the glyphosate-tolerant CP4 EPSPS protein were determined primarily using a qualitative weight-of-evidence approach. Effect and exposure information for humans, livestock, and wildlife (such as mammals, birds, and fish) was obtained from the scientific literature. Other information was obtained from regulatory reports and submissions.

Mutated protein risk

As with the CP4 EPSPS protein, risks for the mutated imidazolinone-tolerant AHAS protein were determined using a qualitative weight-of-evidence approach. However, effect and exposure information for the mutated AHAS protein is not available in the scientific literature. Further, because it is a mutagenic trait and not a genetically engineered trait, regulatory approvals are not required in the U.S. The regulatory status of imidazolinone-tolerant wheat also limits the availability of public information. In Canada, imidazolinone-tolerant wheat is regulated as a novel trait by the Canadian Food Inspection Agency (CFIA) and Health Canada. Therefore, we used the decision documents produced by these two agencies for our risk assessment.

Results

Both glyphosate and imazamox presented lower human health and ecological risks than many other herbicides associated with conventional wheat production systems. The differences in risks were most pronounced when comparing glyphosate and imazamox to herbicides currently with substantial market share. Current weight-of-evidence suggests that the transgenic CP4 EPSPS protein present in glyphosate-tolerant wheat poses negligible risk to humans, livestock, and wildlife. Risk for mutated AHAS protein in imidazolinone-tolerant wheat most likely would be low, but there were not sufficient effect and exposure data to adequately characterize risk. Environmental risks for herbicides were more amenable to quantitative assessments than for the transgenic CP4 EPSPS protein and the mutated AHAS protein.

An important caveat emerges from our work: Tier 1 risk assessment approaches have limited value for accurate quantifications of risk because of their simplistic hazard and exposure assumptions. These assumptions, which are highly conservative and err on the side of environmental safety, typically are used

for highlighting significant versus negligible risks during preliminary decision-making and not for determining actual site-specific risks. Therefore, our results should not be used as representations of "actual" risks. To determine more realistic risks, higher-tier assessments for these technologies should be used. However, we believe quantitative and qualitative tier 1 approaches are valuable for making direct comparisons between environmental stressors.

In our work, environmental risks for herbicides were more amenable to quantitative assessments than for the transgenic CP4 EPSPS protein and the mutated AHAS protein. Because of specificity and familiarity with their native counterparts, evolving regulatory requirements, and the fact that they are not pesticides, these proteins do not have the same completeness of toxicity testing data as herbicides. We believe it is important that minimum effect and exposure data (such as acute mammalian toxicities to altered or inserted proteins) are generated and made publicly available for all novel plant traits, including non-genetically engineered approaches. These data would allow for independent, third-party tier 1 assessments of risk and proper communication of those risks to the public.

Although our assessment was not comprehensive, we believe the approach demonstrates the potential risk-risk tradeoffs when implementing the newer biotechnologies. We are currently applying similar comparative risk approaches to plant-based pharmaceuticals. These types of comparative biological risk assessments add valuable information, which subsequently aid regulatory and public decision-making about biotechnology.

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Possible Health Aspects of Horizontal Transfer of Microbial Transgenes Present in Genetically Modified Crops

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Since the first large-scale introduction of genetically modified (GM) crops a decade ago, the global area cultivated with these crops has undergone a continuous increase, amounting to a total of 90 million hectares in 2005.[1] For comparison, this area equals the national sizes of Portugal, Spain, and Italy together. Many of the "foreign" genes that have been introduced into these crops, i.e., the transgenes, are derived from microbial sources. As explained below, the issue of their potential transfer to other organisms was addressed in a recent article published by our group.[2]

Long before the first introduction of GM crops, international organizations like the Food and Agriculture Organization (FAO), World Health Organization (WHO), and Organization for Economic Co-operation and Development (OECD), had been promoting international consensus on how to assess the safety of such crops. An internationally harmonized approach of comparative safety assessment was thus formulated in which the GM crop is compared to a conventional counterpart with a known history of safe use (reviewed in [3]).

Usually, this comparison entails a description of the genetic modification, such as the nature

of the DNA used and the function of the transgenes and encoded proteins, as well as of agronomic and phenotypic traits and composition. Based upon the differences thus identified, a strategy for further safety assessment can be chosen. Given the wide variety in characteristics of both the host crops and the transgenes, this approach entails decisions on a case-by-case basis, rather than a "cook book" with standard recipes.

Issues that are commonly addressed during the regulatory safety assessment of GM crops include:

- Molecular characteristics, such as the introduced DNA, its integration site (e.g., flanking DNA), and its expression;
- Comparison of agronomic and/or phenotypic characteristics and composition of key macro- and micro-nutrients, anti-nutrients, and toxins;
- Unintended effects that might have arisen from the genetic modification;
- Potential toxicity of newly introduced proteins and of possible changes in the host crop itself, which may have been caused by the genetic modification;
- Potential allergenicity of newly introduced proteins, i.e., the likelihood that they may cause allergies in consumers of food containing GM crops, and possible changes in the intrinsic allergenicity, if any, of the host crop that may have been caused by the genetic modification;
- Nutritional characteristics of the GM crop, which have been already partially addressed by the compositional analyses, and which may also entail animal feeding studies;
- Horizontal gene transfer, i.e., the "natural" genetic modification of organisms other than the crop itself with the newly introduced DNA, for example after the transgene has been released from the crop during processing or digestion. This would require, among others, the uptake of the released DNA by cells of the other organism and also the successful incorporation of this DNA into the new host's genetic material and its expression. Consideration is given to the likelihood of such a transfer to pathogenic microbes in the human intestines, and if it occurred, which consequences it would entail for consumers' health.

In 2003, the activities on international consensus building culminated into the establishment of Codex Alimentarius' guidelines on the conduct of safety assessment of foods derived from genetically modified plants and micro-organisms.[4] Codex Alimentarius standards, guidelines, and other documents are important because they serve as reference for international trade disputes over the safety of internationally traded foods under the international agreement on sanitary and phytosanitary standards (SPS).

Horizontal gene transfer is one of the important issues addressed during the safety assessment of GM crops. In the Codex Alimentarius guidelines, the focus of the assessment of this topic is restricted to the potential transfer of antibiotic resistance marker genes and the consequences thereof. These marker genes are used to facilitate the process of genetic modification. This is done by co-introducing the gene of interest with an antibiotic resistance gene into the DNA of a crop cell. Those cells that have been successfully modified can be selected based upon their ability to sustain on culture media containing the pertinent antibiotic, to which non-modified cells are sensitive. Antibiotic resistance marker genes therefore do not serve a purpose in the GM crop itself.

Antibiotic resistance currently is a matter of great priority to health care, as evidenced, for example, by the attention devoted to this issue by organizations like the WHO. For example, popular media give accounts of the dissemination in hospitals of antibiotic-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA). In general, the spread of antibiotic resistance is considered to be linked to the way that antibiotics are used, among other factors.

During the safety assessment of GM crops, the possibility of the transfer of antibiotic resistance

genes that have been introduced into GM crops is considered. The European Food Safety Authority's Scientific Panel on Genetically Modified Organisms recently issued an opinion on antibiotic resistance genes.[5] This opinion, among others, proposed a categorization of the antibiotic resistance genes into three categories based on the clinical importance of the antibiotic, the natural prevalence of resistance to the same antibiotic in nature, and the likelihood of transfer. Only antibiotic resistance genes that fall into the first category of this scheme, such as the kanamycin resistance gene *nptII*, are recommended to be allowed for use in GM crops that are to enter the market.

In practice, however, regulatory safety assessments do not limit the scope of potential transfer of transgenes from GM crops only to antibiotic resistance. These assessments also address other potential effects of transgenes, including pathogenicity. The potential impacts of gene transfer on health and the environment in a broad sense are considered by European Union guidelines.[6,7]

Similar to antibiotic resistance, literature reports indicate that characteristics associated with pathogenicity have been exchanged between microorganisms like *Escherichia coli* and *Salmonella enterica*, such as through transfer of DNA fragments containing "pathogenicity islands." A wide array of biochemical characteristics are known to be involved in the pathogenicity of microorganisms, such as the formation of adhesion molecules that bind to host cells, enzymes that facilitate entrance into host cells, self-sufficiency for some nutritional compounds, and "quorum sensing" within groups of micro-organisms.

Various mechanisms by which DNA is horizontally transferred between microorganisms are known to exist in nature, including transfer after conjugation between bacteria, transduction by bacteriophages, and transformation by free DNA. Potential transfer of transgenes from GM crops to microbes in the gastro-intestinal tract likely proceeds through a process in which competent cells are transformed with free DNA. As stated above, this can occur after the DNA of the GM crops has been released from its host cells, for example during digestion.

Various factors influence the likelihood that transfer of DNA from a GM crop to a recipient bacterium will occur and become productive. One of these factors is the level of the bacterium's competence, i.e., the physiological state of a bacterial cell during which it can bind, take up, and recombine DNA molecules. The outcomes of a number of studies indicate that the most likely mechanism by which DNA is transferred from GM crops to microorganisms is by homologous recombination. This means that the recipient microorganisms should already contain sequences that are sufficiently similar ("homologous") to the incoming foreign DNA, such that they can align with each other and allow for integration of the latter.

Finally, plant genes and microbial genes differ with respect to preferred base composition of the codons. Plant genes also have other features that differ from microbial genes, such as introns, which do not occur in bacterial sequences, and different types of regulatory sequences.

On the one hand, based on these considerations, which have been reviewed in more detail elsewhere,[8] it appears that transgenes of microbial origin carry an enhanced likelihood of being transferred from GM crops to microorganisms. Genetic modification allows for the introduction of foreign genes from one organism into another, unrelated organism. As a result of this, many of the GM crops currently on the market contain transgenes of microbial origin, such as enzymes metabolizing herbicides obtained from soil microorganisms or insecticidal proteins obtained from *Bacillus thuringiensis*.

In our review,[2] we focused on transgenes of microbial origin other than antibiotic resistance genes that are present within GM crops approved by the regulatory authorities of the European Union, United States of America, Canada, Australia, and New Zealand. A number of factors that influence the transfer of these transgenes, as well as the potential impact of

such a transfer on the health of consumers, were considered. For each gene studied, these factors, if applicable and information available, included:

- Occurrence and pathogenicity of the microorganism from which a given gene has been obtained;
- Natural function of the gene;
- Prevalence of the gene in other microorganisms;
- Geographical distribution of the gene;
- Similarity of the original gene and codon-modified transgene to genes in other microorganisms. For this purpose, DNA sequences were compared using the FASTA algorithm. A stringent threshold for similarity was used. In addition, we checked whether the aligned sequences would have two identical stretches of DNA of at least 20 contiguous base pairs each, which is considered the minimum required for homologous recombination. For many transgenes, the actual sequences introduced into GM crops are treated as confidential information and are thus not publicly available. A high degree of similarity may be indicative both for the background presence of the gene in nature, and for the likelihood of transfer by homologous recombination;
- Known horizontal gene transfer activity of the gene. Has this gene previously been transferred in nature?
- Selective conditions and environments, e.g., does the gene confer a selective advantage to its host? If yes, persistence of the transferred gene may be more likely.
- Possible effect of the transgene on the pathogenicity or virulence of its host.

None of these single items can be considered completely predictive for adverse effects and therefore a combination of factors has to be considered in a "weight of evidence"-based approach. Based upon these considerations, a conclusion was formulated for each gene as to whether its transfer from GM crops would be likely to have any adverse health effects in consumers. In total, 20 microbial transgenes were considered, including five that are linked with herbicide resistance, three with hybrid breeding through male sterility, two with prolonged fruit ripening, two linked with markers for genetic modification, and eight with insecticidal properties. The genes with insecticidal properties all encoded Cry proteins from *B. thuringiensis*.[2]

It was concluded that none of these cases raises safety concerns. However, a number of conspicuous findings were made. For example, the native forms of a number of genes appeared to have been transferred horizontally in nature. In some cases, this transfer was postulated by other authors based on sequence similarities between genes from different species, or the ability to transfer plasmids between them under laboratory conditions.[2] This pertained, for example, to the *uidA* transgene from *E. coli* encoding β-glucuronidase, which is used as a marker enzyme in GM crops based on its ability to form a blue color under test conditions. Similar genes with bacterial rather than fungal sequence characteristics were found to occur in moulds residing in soils. The authors of this particular study[9] concluded that the transferred gene would allow the recipient microorganisms to utilize glucuronide compounds, which are formed, for example, in the liver of animals and excreted through feces and urine. The transferred gene would thus have conferred a selective advantage to its recipient in soil.

Another case of selective advantage in soil conditions was that of the 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene, which has been isolated from a soil isolate of *Pseudomonas* and introduced into GM tomatoes to suppress ethylene synthesis and thereby delay ripening. It has been observed that this gene is expressed in soil microorganisms colonizing plant roots and that its activity is associated with increased root formation.[10] We therefore postulated that the transfer of this gene may confer a selective advantage to recipient microorganisms in the vicinity of plants producing ACC.

It should be noted that the data on the original sequences from the native hosts may represent

a "worst case" situation. This is because in GM crops the transgene sequences may have been optimized for expression in plants. As stated above, plant genes have a number of features that are different from bacterial genes, which decrease the likelihood of effective transfer and expression of plant genes to bacteria.

In conclusion, it was recommended to include the abovementioned considerations in safety assessments of GM crops carrying transgenes other than the ones already reviewed in the current survey[2].

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Mitigating Transgene Flow from Crops

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Two general approaches are used to deal with transgene flow: containment of the transgenes within the transgenic crop; or transgenic mitigation of the effects of the primary transgenic trait should it escape. Most containment mechanisms severely restrict gene flow only in one direction. Gene flow (leakage) is

inevitable even in that direction, allowing spread through the population of undesired species, unless mitigated.

Limitations to containing transgene flow

Several molecular mechanisms have been proposed to contain transgenes by preventing introgression to relatives *via* pollen. These containment strategies can either suppress gene outflow from the crop, or protect the crop from inflow from wild or weedy relatives.[1] The proposals to integrate the transgene in the plastid or mitochondrial genomes do not preclude the relative from pollinating the crop and then acting as the recurrent pollen parent. Claims of no paternal inheritance of plastome-encoded traits have been not substantiated, indeed maternal inheritance is leaky. Tobacco and other species typically transmit transplastomic traits via pollen at a frequency of 10^{-3}–10^{-4} in laboratory experiments. A large-scale field experiment utilized a *Setaria italica* (foxtail millet) with chloroplast-inherited atrazine resistance (bearing a nuclear dominant red leaf base marker) crossed with five different male sterile yellow- or green-leafed herbicide susceptible lines. Chloroplast-inherited resistance was pollen transmitted at a 3×10^{-4} frequency in >780,000 hybrid offspring.[2] Thus, chloroplast transformation is probably unacceptable for preventing transgene outflow, unless stacked with additional mechanisms.

Other molecular approaches suggested for crop transgene containment such as seed sterility, utilizing the "genetic use restriction technologies" (GURT), and recoverable block of function do not prevent transgene outflow from the crop in seed propagation fields, just inflow from the relative.[see 1]

Risk can be reduced by stacking containment mechanisms together, compounding the infrequency of gene introgression, but once it occurs, the new bearer can disperse the transgenes with just a small fitness advantage throughout the population.[1]

Mitigating establishment of 'leaked' transgenes

The spread of genes can be mitigated by maintaining the fitness of recipients below the fitness of the wild type. A concept of "transgenic mitigation" (TM) was proposed in which mitigator genes are tandemly linked to the desired primary transgene, which would reduce the fitness of hybrids and their rare progeny, considerably reducing risk.[see 1] This TM approach is based on the premises that: 1) tandem constructs act as tightly linked genes with exceedingly rare segregation from each other; 2) the TM traits chosen are neutral or favorable to crops, but deleterious to non-crop progeny; and 3) individuals bearing even mildly harmful TM traits will remain at very low frequencies in weed/wild populations because weeds typically have a very high seed output and strongly compete among themselves, eliminating even marginally unfit individuals. Thus, if the primary transgene of agricultural advantage is flanked in a tandem construct by TM gene(s) such as dwarfing, uniform seed ripening, non-shattering, anti-secondary dormancy, or non-bolting genes, the overall effect would be deleterious after introgression into relatives—the TM genes will reduce the competitive ability of the rare transgenic hybrids such that they cannot compete and persist in low frequencies in agroecosystems.

We used tobacco as a model to test the TM concept: a tandem construct was made containing an *ahas*^{R} (acetolactate synthase) gene for herbicide resistance as the primary desirable gene, and the dwarfing Δ*gai* (gibberellic acid-insensitive) mutant gene as a mitigator[3]. Dwarfing would be disadvantageous to the rare weeds introgressing the TM construct, as they could no longer compete with other crops or with fellow weeds, but is desirable in many crops, preventing lodging and producing less straw with more yield. The dwarf and herbicide resistant TM transgenic hybrid tobacco plants were more productive than the wild type when cultivated separately. They formed many more flowers than the wild type, which is indicative of a higher harvest index.[3] Conversely, the tobacco TM transgenics were weak competitors and highly unfit when co-cultivated with the wild type in ecological simulation competition experiments, and none set seeds at close spacing, even when 75% of the plants were TM and 25% wild type, in a replacement series.[4]

Mitigation in a *Brassica* crop and related weed

We inserted the same construct into oilseed rape (*Brassica napus*) and tested the selfed progeny,[5] as well as hybrids and backcrosses with the weed *Brassica campestris* = *B. rapa*.[6] When cultivated separately, the dwarf transgenic oilseed rape grew slightly slower than the non-transgenic (**Fig. 1**), but produced > 50% more seed at the expense of the stem tissue (**Table 1**). When the TM transgenic oilseed rape plants were co-cultivated in competition with the wild type, they were suppressed and unable to grow normally (**Fig. 1**), and hardly set seed (**Table 1**) because they were so unfit to reproduce.[5] The TM hybrids with the weeds and their further backcrosses to the weeds were also exceedingly unfit and unable to compete with wild type weed.[6] In any rotational system, where the selector herbicide will not be used in the following crops, the TM offspring will be out-competed by non-transgenic cohorts and other species.

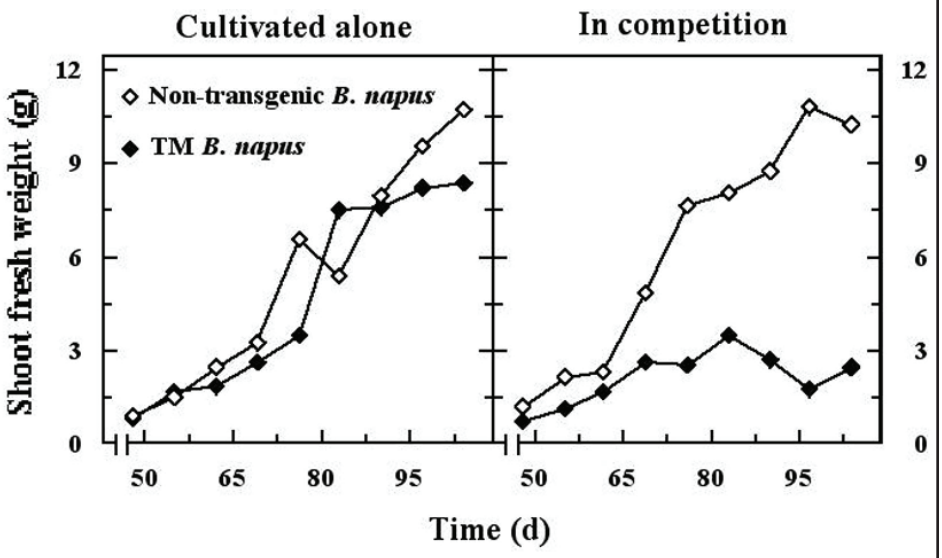

Figure 1: Suppression of growth of TM (transgenically mitigated) oilseed rape carrying a dwarfing gene in tandem with a herbicide resistance gene (closed symbols) when in competition with wild type (open symbols) (right panel), and their normal growth when cultivated separately without herbicide use (left panel) at 3-cm spacing.[5]

Thus, transgenic mitigation is clearly advantageous to a crop grown alone, while disadvantageous to a crop-weed hybrid living in a competitive environment. If a rare pollen grain bearing tandem transgenic traits hybridizes with a relative, it must compete with multitudes of wild type pollen to produce a hybrid. Its rare progeny must then compete with more fit wild type cohorts during self-thinning and establishment. A small degree of unfitness encoded by the TM construct would cause elimination of most progeny in all future generations as long as the primary gene provides no selective advantage and the linked gene confers unfitness.

Field studies are needed with crop/weed pairs to further evaluate risks with mitigation. The rare

Table 1 Transgenic mitigated oilseed rape has high productivity but low relative competitive fitness in hybrids with wild type and with a related weed

Biotype	Productivity grown alone	Relative fitness in competition with	
		B. napus NT	*B. rapa*
B. napus NT	320[b]	-	4.3
B. napus TM	503[a]	0.10	2.8
B. rapa	119[d]	-	-
F_2 hybrids (*B. rapa* X *B. napus* NT)	213[c]	-	0.9
F_2 hybrids (*B. rapa* X *B. napus* TM)	75[e,d]	-	0.02
F_2 BC_1 [*B. rapa* X (F_1 hybrids NT)]	63[e,d]	-	0.2
F_2 BC_1 [*B. rapa* X (F_1 hybrids TM)]	26[e]	-	<0.01

Productivity - seed weight/plant (mg); relative fitness - ratio of transgenic (TM) seed weight/plant to non-transgenic (NT). Different letters indicate different LSD values at $P \leq 0.05$. Sources: refs.[5,6]

hybrid offspring from escaped pollen bearing transgenic mitigator genes should not pose a dire threat, especially to wild species outside fields, as the amount of pollen reaching the pristine wild would be minimal, and competition with the wild maximal.

Special situations require special mitigators

The persistence of pharmaceutical transgenes in maize and their flow by pollen to neighboring fields could be mitigated by a tandem construct with "shrunken seed" loci (RNAi of sugar transformation to starch). Volunteer shrunken seeds and hybrids in nearby fields (discarded during harvest) cannot overwinter. Phytoremediation of soils could utilize the overexpression of cytokinin oxidase; the phenotypes have reduced shoot systems (unfitness to compete) but have faster growing, more extensive root systems, which are better for extracting toxic wastes.[7] Vegetatively propagated trees and other species could be rendered male and female sterile. Biennial and other bolting crops can be made non-bolting by interfering with gibberellic acid production, using the hormone to allow flower production for seed.[1]

Thus, transgenic mitigation systems can probably be conjured for any need to mitigate the effects of gene flow. The greatest security will be obtained when the gene of choice is flanked on either side by TM constructs, and is stacked with a containment system, or is in a male sterile background.[8]

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