

## Environmental risks of genetic engineering

E. Ann Clark

Plant Agriculture Department, University of Guelph, Guelph, Ontario, Canada N1G 2W1  
(e-mail: eaclark@uoguelph.ca)

*Key words:* gene flow, genetic modification, GMO, transgenic

### Summary

Before release into commerce, genetically engineered organisms are first assessed for possible risks, including risks to the environment. The present paper first identifies the environmental risks recognized by regulators, and reviews the parameters considered predictive of risk. Recent field-scale studies suggest opportunities for improvement of the environmental risk assessment process. Risks unique to genetically engineered crops – if any – could pertain to the specific traits chosen for commercialization and to unintended trait expression caused by the process of transgene insertion itself. Both the standard against which to compare genetically engineered traits and the scale of exposure need to be considered when assessing environmental impact. Evidence of environmental risk in the recognized areas of weediness on agricultural land, invasiveness of unmanaged systems, and non-target impacts from *Bacillus thuringiensis* (Bt) maize is presented. Targeted, statistically sound, rigorously conducted, multi-trophic studies analogous to the Field Scale Evaluation trials recently completed in the UK are needed to clarify the many questions which remain unanswered.

*Abbreviations:* APHIS, Animal and Plant Health Inspection Service; Bt, (*Bacillus thuringiensis*); CFIA, Canadian Food Inspection Agency; FSE, Farm Scale Evaluation; GM, genetic modification; GMO, genetically modified organism; GE, genetic engineering; GMHT, genetically modified herbicide tolerant; HT, herbicide tolerant; RR, Roundup Ready

### Introduction

Potential impacts of genetically modified organisms (GMOs) on the environment have been addressed in several authoritative reviews (Rissler & Mellon, 1996; Traynor & Westwood, 1999; Wolfenbarger & Phifer, 2000; Letourneau & Burrows, 2002; NRC, 2002), to which the reader is referred for further information. The present paper seeks first to identify those environmental risks which are recognized for GM crops, and then reviews the parameters considered predictive of the identified risks. Evidence from recent studies is then presented with the aim of suggesting possible improvements to contemporary risk assessment. Risks unique to GM crops are viewed from two perspectives: the specific traits chosen for commercialization, and the potential for inadvertent trait expression due to the

process of transgene insertion. Potential GM impacts on the environment, specifically weediness on agricultural land, invasiveness into unmanaged environments, and non-target impacts from *Bacillus thuringiensis* (Bt) maize, are discussed.

The terms GE (genetic engineering), genetic modification (GM), and transgenic will be used interchangeably to refer to the insertion of DNA (or RNA) into the genome of a host organism. The resultant transgenic organism will be referred to as a GMO.

### Environmental risk assessment

Assessment of the potential risks of GMOs is currently limited to the intended target traits, which are predominantly herbicide tolerance (HT), pesticidal plants (Bt),

or both. Possible effects from non-target traits will be discussed later (see Risks from inadvertent trait expression). Of interest here is what constitutes an environmental risk, the predictive value of the parameters currently used to assess environmental risk from target traits, and how recent evidence of multi-trophic effects, field-scale impacts, and future novel GM traits could inform refinements to risk assessment.

#### *Risk and risk prediction for target traits*

Which environmental risks are recognized for GM crops, and which criteria are currently used to assess risks? In one of the first comprehensive analyses of its kind, Rissler and Mellon (1996) identified six types of environmental risks, including weediness, outcrossing to wild plants, and non-target impacts, which could cascade into broader ecological impacts. Risks from crops engineered to produce viruses which may themselves create more virulent or widespread viruses were also noted. They expressed particular concern about threats to centers of diversity of crop species.

The Canadian Food Inspection Agency (CFIA) is the regulatory body responsible for assessing environmental risks of GMOs in Canada. The potential risks acknowledged by the CFIA are (a) weediness on agricultural land, (b) invasiveness into unmanaged habitats, (c) potential for gene flow to wild relatives, (d) plant pest potential, (e) potential impact on non-target organisms, and (f) potential impact on biodiversity. A similar array of risks is recognized by Animal and Plant Health Inspection Service (APHIS) in the United States (NRC, 2002).

The case example of event T120-7, a glufosinate ammonium tolerant sugar beet (*Beta vulgaris*) (CFIA, 2002), illustrates the CFIA approach to environmental risk assessment in GM crops. For this particular submission, the CFIA accepted seed germination, plant vigor, flowering period, time to maturity, and overwintering capacity, as well as field observations of disease and pest susceptibilities as predictive of environmental risk. Toxicological studies were also reported for the purified gene product of the target transgene. As in all submissions to date, the tested gene product was expressed in microbial culture. Possible homology between the nucleotide sequence of the target gene(s) and of the amino acid sequence of the gene product with those of known toxins and allergens was also examined (CFIA, 2002).

Based on the comparability of the transgenic line with its unmodified counterpart in these parameters, the

CFIA concluded that “Tolerance to glufosinate ammonium will not, in itself, render sugar beet weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified,” and furthermore, that because the “novel traits have no intended effects on weediness or invasiveness, all sugar beet lines derived from event T120-7 (will) have no altered weed or invasiveness potential compared to currently commercialized sugar beets. . .” (CFIA, 2002). The decision to authorize the unconfined release of not just event T120-7 lines but all subsequent lines and hybrids presumed that the aforementioned suite of measured or inferred parameters provides sufficient information to assess potential environmental risk, and further, that risk would not change through subsequent crossing.

The absence of detectable environmental harm from the millions of hectares of GM crops which have been sown annually for the last several years would support the adequacy of contemporary risk assessment processes in both the United States and Canada. However, CBCGEO (2004) regarded such claims as unscientific in the absence of systematic monitoring for environmental impacts.

#### *Improving assessment of target trait risks*

Gauging possible environmental impacts of a crop cultivar is a novel exercise, whether or not the cultivar has transgenic traits, because of the difficulty of identifying, *a priori*, those attributes which are predictive of weediness, invasiveness, gene flow, pestiferousness, or impacts on either non-target organisms or biodiversity. Risks specific to GM crops – if any – are even more complicated, given the scarcity of research funding devoted to this task. Mellon and Rissler (2003) noted that the 1990 US Farm Bill allocated 1% of the USDA budget on biotechnology to risk assessment research in the United States, an amount which increased to 2% in 2003. Gaps in the research available to assess environmental impacts, including containment, are commonly cited by reviewers (Wolfenbarger & Phifer, 2000; Letourneau & Burrows, 2002, NRC, 2002; CBCGEO, 2004).

The NRC (2002) distinguished *hazard* – a potential harm – from *risk* – the likelihood that a harm will actually occur. They identified four categories of environmental hazard from GM crops: (a) those caused by the movement of the transgene and its subsequent expression in other hosts; (b) those deriving from the transgenic crop itself; (c) non-target effects other than (b); and (d) resistance in target populations. Most recently,

the CBCGEO (2004) proposed a more encompassing process, starting with hazard identification, risk analysis and mitigation, followed by monitoring and remediation. The NRC concluded that, had such a process been in place, it would have avoided the containment problems which occurred with StarLink maize (*Zea mays*) (Smyth et al., 2002; Clark, 2004; various cited in CCGEO, 2004).

Based on a survey of the ecological literature, Purrington and Bergelson (1995) proposed that a suite of 14 parameters would be necessary to assess risk of weediness, including seed viability, dormancy, production and dispersal; growth rate and period; clonal reproduction; lifetime survivorship; competitiveness; geographic range; pollen flow and performance; fitness of hybrids with other cultivars; fitness of hybrids with wild species. They also supported assessment not just of the transgenic line but also of reciprocal crop/weed hybrids, even in Canada or the United States that may have no natural wild relatives. In contrast, the CFIA protocol bypasses the issue of gene flow to wild relatives for crops which did not evolve in Canada. In the case of event T120-7 sugar beet, for example, they reasoned that because "... there are no wild relatives in Canada that can hybridize with sugar beet ... gene flow from sugar beet lines derived from event T120-7, to wild relatives is not possible in Canada." Purrington and Bergelson (1995) suggested that a more encompassing approach might be prudent, given the implications of global crop movement to countries with natural wild relatives or landraces.

Rissler and Mellon (1996), Mack et al. (2000), and NRC (2002) questioned the predictive value of trait lists, such as Baker's List for plant invasive ability, which are reflected in current risk assessment protocols, because of the large number of exceptions (however, see also Warwick et al., 1999). Williamson (1993) argued that ecological attributes would not necessarily be predictive of that small proportion of GMOs that may become invasive. Gidding (1999) cited evidence of the difficulty of relating invasiveness to biological, genetic and/or environmental traits. The question of which traits are predictive of environmental risk remains open.

Various contributors have offered stepwise flowchart or decision tree approaches to improve the current process (Scientists Working Group on Biosafety, 1998; Gidding, 1999; Letourneau et al., 2002; NRC, 2002). Marvier (2002) emphasized the importance of a standardized and defensible methodology in assessing environmental risk. She

demonstrated that small sample size, limited replication, and inconsistencies in the pattern, duration, and extent of exposure, both among GM submissions and between test and natural organisms, have detracted from the statistical power of studies on ecological risks of GMOs. In analyzing APHIS assessments for specific GM crops, the need for improvement in sample size and experimental method was also noted by the NRC (2002).

A purposeful array of questions, using scientifically sound methods, would help to assess the potential for environmental risk from GMOs. The first commercial-scale study to examine impacts on non-target organisms and biodiversity (CFIA issues (e) and (f)) under realistic conditions was the Farm Scale Evaluation (FSE) trial in the UK. Potential impacts of genetically modified herbicide tolerant (GMHT) cultivars of three spring-sown crops – canola (*Brassica napus*), sugar beet, and maize – were evaluated for 3 years starting in 2000 (Squire et al., 2003). A split-field design was used on 60–70 farms per crop to compare the effects of conventional and GMHT methods for weed control on farmland biodiversity, focusing on weeds as well as on soil surface- and vegetation-dwelling invertebrates. Analysis of the FSE trial placed the changes due to GMHT cultivars within the context of broader impacts of agriculture on the countryside. Various FSE contributions followed GMHT impacts through trophic levels, connecting the crops and weeds with the herbivore, predator, and parasitoid communities in arable land and field margins (Brooks et al., 2003; Firbank et al., 2003; Houghton et al., 2003; Hawes et al., 2003; Heard et al., 2003).

Working in the same region, Dewar et al. (2003) had inferred that GMHT sugar beet would help declining bird populations by allowing greater early season weed and, hence, insect growth. However, the FSE trial showed that the greater effectiveness of GMHT technology in controlling both weeds and the weed seed rain reduced most insect species, including butterflies (Brooks et al., 2003; Houghton et al., 2003), and, by inference, birds in sugar beets and canola although not in maize (Heard et al., 2003). As envisioned by Rissler and Mellon (1996), GMHT responses ramified through trophic levels (Hawes et al., 2003) having a more adverse effect on biodiversity than conventional methods of weed control for two of three tested crops. However, the greatest diminution in natural biodiversity was attributed to longitudinal trends in agricultural intensification, and specifically, the homogenization of the UK agricultural landscape on a regional basis.

Firbank (2003) emphasized that the results of the multi-year, multi-site FSE trial pertained to a single trait – HT – assessed through a limited range of biodiversity indices associated with weed control, in a specific, intensively farmed region. The ramifying multi-trophic impacts documented in the FSE may, for example, be of less concern in regions less intensively cropped, with larger unmanaged buffer areas. The potential for other ecological impacts, such as those from pesticidal (Bt) or pharmaceutical plants on the evolution of wild progenitors in a center of origin, awaits further study. Nonetheless, the questions addressed by the FSE study reveal insights into multi-trophic GM risks, which could materially improve the predictive value of environmental risk assessment.

Another field-scale trial bearing on issues considered in the CFIA protocol involved long distance pollen-mediated gene flow from RR creeping bentgrass (*Agrostis stolonifera*) to both sown and wild relatives (Watrud et al., 2004). They monitored crossing in both 178 transplanted sentinel *A. stolonifera* plants and 79 resident plants (30 for *A. stolonifera*; 39 for *A. gigantea*, and 10 for *Polypogon monspeliensis*) – all of which are cross-compatible. Monitored plants were distributed in elongated collection zones extending 4, 8, 16, and 24 km away from eight sources RR fields – totalling 162 ha – located within a 4453 ha GM bentgrass control district. Sampling design was premised on maximal pollen viability of 3 h and prevailing winds of 10 km h<sup>-1</sup> from the north and northwest. The zone of documented GM pollen movement extended to 21 and 14 km in sentinel and resident plants, respectively, encompassing 310 km<sup>2</sup> over both agronomic and unmanaged landscapes. Progeny able to withstand two sprays of Roundup were obtained from 54 and 53% of the sentinel and resident *A. stolonifera*, respectively, 33% of the *A. gigantea*, and 0% of the *P. monspeliensis* plants. Rates of crossing exceeded those previously reported, apparently due to the scale of the source plots.

Creeping bentgrass is classed as both competitive and ruderal, spreading via laterally invasive root and stolon growth as well as dispersal through seeds (Watrud et al., 2004). Gene flow to wild relatives was demonstrated, but possible impacts on biodiversity from gene flow in GM perennial grasses remain to be studied. While the RR trait would not be expected to enhance fitness in the wild, it could affect control of both creeping bentgrass and its wild relatives in managed forest, turf, or agronomic settings, as discussed by Martinez-Ghersa et al. (2003). The geographic scale of

this study clearly informs the issues of gene flow to wild relatives and plant pest potential.

#### *Future GM traits*

The issue of potential environmental impact pertains to more than the HT and Bt traits, which currently dominate GM hectareage. A wide range of plant, animal, and microbial applications is in varying degrees of readiness for commercialization, as tabulated by the Pew Initiative on Food and Biotechnology (2001) and more recently by the CBCGEO (2004). Industrial/pharmaceutical applications include antibodies, avidin, and edible vaccines as well as industrial enzymes and plastics. Apples modified to contain a chitinase gene for control of apple scab (*Venturia inaequalis*) have been field tested, as has Bartlett pear engineered for resistance to fireblight (*Erwinia amylovora*), and RR-poplar. Many pending traits have never existed in the environment on a commercial scale, resulting in potentially novel ecological and evolutionary impacts. Thus, while HT is unlikely to affect fitness in the wild, Bt and other more adaptive traits may require additional refinements to environmental risk assessment.

In summary, environmental risk assessment protocols have been in place for several years and have served effectively in pre-screening GM crops prior to commercialization. However, both the NRC (2002) and the CBCGEO (2004) recommended systematic post-release monitoring to validate pre-release risk assessment decisions. New research, accumulating evidence from field-scale trials, and an array of pending novel GM traits raise additional issues for future improvements to the risk assessment process

#### **Risks from inadvertent trait expression**

Do GMOs pose unique environmental risks? Apart from the possible ecological effects from the wholly novel traits enabled by GM technology, a predilection for unpredictable side effects from transgene insertion could pose unique risks. Dale et al. (2002) were unable to find evidence that GM crops differ innately from non-GM crops. The NRC (2002) concluded that there was no *a priori* reason to presume that GM crops pose risks not found in conventionally bred crops, particularly given the grey areas of mutagenesis, wide crossing, and embryo rescue which are included under conventional breeding. Even the superior weed control expressed by

GMHT sugar beet and canola in the FSE trial (Firbank, 2003) could have occurred through non-GM as well as GM herbicide tolerance. For example, mutagenesis produced the non-GM Clearfield trait that confers tolerance to imidazolinone herbicides. Al Khatib and Miller (2000) reported the spontaneous occurrence of a natural mutant conferring the same tolerance in sunflower (*Helianthus annuus*). The NRC (2002) also provided several examples to show that unintended side effects can occur from conventional breeding as well as from GM.

Nonetheless, GE – by definition – relies on genetic changes of a type and scale unprecedented in nature. Insertion of alien DNA did not originate with GE, but rather, has occurred throughout evolutionary time. The widespread occurrence of a suite of sophisticated intracellular mechanisms for continually monitoring DNA integrity and silencing intruders (Meyer, 1996; Hall et al., 1998; de Neve et al., 1999; Demeke et al., 1999; Kumpatla et al., 1998; Chandler & Vaucheret, 2001) suggests that alien gene insertion is both a natural process and one that apparently poses significant risks to genome integrity. That circumventing these ubiquitous mechanisms is an essential prerequisite to GE suggests caution.

#### *Unrelated gene expression*

Genetic modification – and the protocol for environmental risk assessment of GMOs – dates from an era when genes could reasonably be expected to perform the same function in a new genome as in the original host. Accumulating evidence illustrates that gene-to-gene, gene-protein, and gene-to-environment interactions affect expression in ways that are still not completely understood.

To increase fermentation, Inose and Murata (1995) inserted multiple copies of an existing gene into yeast (*Saccharomyces cerevisiae*) and inadvertently elicited not just a 3-fold increase in phosphofructokinase, an enzyme in the glycolytic pathway, but also a 40–200-fold increase in methylglyoxal – a toxic and mutagenic substance. Genetically modified tobacco (*Nicotiana tabacum*) intended to produce gamma-linolenic acid also produce octadecatetraenic acid, a highly unsaturated, industrially important product which does not naturally occur in tobacco (Reddy and Thomas, 1996). Bergelson et al. (1998) took a naturally occurring mutant gene conferring chlorosulfuron resistance (*Csr1-1*) from *Arabidopsis thaliana* and inserted it into non-mutant individuals of the same species. Both transgenic

and mutant individuals expressed the same *Csr1-1* gene, but per plant outcrossing rate changed from 0.3% for mutant fathers to 6% for transgenic fathers – a roughly 20-fold enhancement in outcrossing. Saxena and Stotzky (2001) compared 10 Bt-maize hybrids, encompassing three events (Bt-11, Mon810, 176), with their respective isolines in both field and growth room conditions. Lignin content was significantly higher in Bt isolines for all contrasts, in both field and growth room conditions. In each case, the reported responses were unrelated to the intended traits.

Inability to anticipate which particular genes or metabolic pathways may be affected and how they may respond to transgene insertion means that it is impossible to screen against toxins or other unanticipated by-products. Specifically because they cannot be predicted, unintended effects on the broader environment cannot be assessed prior to commercial release.

#### *Unpredictable agronomic impacts*

The experimentally based concerns identified earlier are corroborated by the apparent vulnerability of commercialized GM technology, and specifically the RR trait which accounts for the majority of the GM hectare, to expression of agronomically important unintended traits. The RR trait, or perhaps the genomic region linked to the RR gene, has been reported to increase susceptibility to sudden death syndrome in soybean (*Glycine max*) caused by the soil pathogen *Fusarium solani* (Sanogo et al., 2000), although Njiti et al. (2003) failed to corroborate this effect. In cotton (*Gossypium hirsutum*), the RR trait has been reported to increase susceptibility to root knot nematode (*Meloidogyne incognita*) (Colyer et al., 2000).

The evidence for RR trait effects on *Sclerotinia* root rot (*Sclerotinia sclerotiorum*) susceptibility in soybean is inconsistent. When compared with untreated controls, glyphosate exerted differing effects on four RR soy cultivars, with disease severity index significantly increasing in two and significantly decreasing in one, while another was unaffected (Nelson et al., 2002). For one cultivar, disease severity was higher in the RR than in its non-RR isolate, when neither was treated with glyphosate. In contrast, Lee et al. (2003) compared defences to *S. sclerotiorum* in a pair of RR and non-RR near isolines of soybean and found that glyphosate did not affect susceptibility to *S. sclerotiorum*. Thus, the postulated effect of either glyphosate or the RR trait itself on disease susceptibility may be cultivar specific.

Compared to non-RR cultivars, water stress reportedly reduced N fixation in RR soybean (King et al., 2001) and promoted fruit abscission in RR cotton (Pline et al., 2003). However, Reddy and Zablotowicz (2003) reported that while application of various glyphosate salts could reduce both nodule biomass and leghemoglobin content, soybean recovered later in the season. In a hand-weeded, multi-site trial, Elmore et al. (2001a,b) demonstrated lower yield in RR than in non-RR soybean isolines, as corroborated on a large scale by Benbrook (2001) and cited by Martinez-Ghersa et al. (2001).

Unintended trait effects may carry over to subsequent crops. Descalzo et al. (1998) noted that glyphosate acts by inhibiting an enzyme of the shikimic acid pathway, of which a major product is phenylalanine. Phenylalanine is a known precursor of compounds associated with disease resistance, such as lignin, flavonoids, and phytoalexins. Deleterious secondary effects on subsequent crops resulted from apparent synergies between pre-plant glyphosate and soilborne pathogens in the genera *Pythium*, *Fusarium*, and *Rhizoctonia*, as cited by Descalzo et al. (1998). When studied, secondary effects were not noted with other herbicides, suggesting a selective effect of glyphosate.

Possible effects of glyphosate-based herbicides on fungal pathogens of wheat (*Triticum aestivum*) were examined in a 4-year study by Fernandez et al. (2003) in Saskatchewan. The study was motivated by the importance of Fusarium head blight (*Fusarium* sp.) in western Canada, the dependence on glyphosate in conservation tillage systems in the region, and by the possible commercialization of a new RR-wheat. Over 659 spring wheat fields, prior application of glyphosate-based herbicides was the crop production factor most closely associated with development of Fusarium head blight in farmers' fields. Hanson and Fernandez (2003) reported that amending agar with any of seven formulations of glyphosate-based herbicides significantly increased growth of both Fusarium head blight and tan spot (*Pyrenophora tritici-repentis*) in culture, irrespective of rate of application. Thus, glyphosate-based herbicides, whether applied to GM crops or in conventional, non-GM usage, may be associated with increased pathogenicity in the following crops. Whether this phenomenon is caused by the herbicide or by the GM crop is arguable, but when the GM trait necessarily invokes use of the herbicide, the risk is the same.

The unintended effects associated with the RR trait may be genetic or physiological in nature, given the

importance of the shikimic acid pathway for both disease resistance and stress tolerance. However, the effects mentioned earlier were detected specifically because they were of agronomic importance, leaving unanswered the question of what other, less visible traits may also have been affected, particularly in food crops.

### Potential for GMO impact on the environment

Of the recognized categories of environmental risk, published work is most readily available for weediness on agricultural land, invasiveness, and for non-target impacts of Bt crops. Qualifying issues include the appropriate comparator for possible GMO risk, and the scale of impact in time and space.

#### *Basis of comparison*

Against what standard should GMO impact be assessed? Virtually all analysts to date have used conventional agriculture as the standard, including regulators (CFIA, 2002; NRC, 2002), designers of the FSE trial (Firbank, 2003), and Dale et al. (2002). The logic of the assumption that the risks posed by traits from conventional breeding provided an 'acceptable' baseline for assessing GM trait risk was challenged by the NRC (2002). The CBCGEO (2004) noted that identifying the appropriate pre-existing technologies against which to compare possible harm from GMOs depends on the context. If the issue is risk within the zone of introduction, then the predominant agricultural practice or best alternative for the region would be an appropriate comparator. However, if the issue is how to reduce environmental risk in the future, then transgenics could be compared with sustainable or organic agriculture (CBCGEO, 2004).

#### *Scale: Time and space*

The time frame over which an environmental impact may develop complicates risk assessment. Mack et al. (2000) noted lag phase which can last for decades or even centuries before an immigrant becomes an invasive species. Marvier et al. (1999) calculated a median interval of 30–50 years between first record of a weed and the onset of widespread infestation. Thus, the impact of adventitiously released transgenes may not be felt for decades, analogous to exotic invaders.

Gidding (1999) also emphasized the importance of environmental stochasticity in the evolution of feral populations, noting that if genes can be maintained in a wild population, chance occurrences of favorable circumstances could transform the host to invasive status. Predictive modeling is effective in assessing potential risk in a predominantly deterministic context, but stochastic processes can be of overriding importance particularly in the longer term. As such, the effectiveness of deterministic models in predicting the invasiveness of GMOs remains to be seen.

Given that alien DNA insertion appears to be a natural if infrequent phenomenon, it could be argued that GE does not pose unique risks. However, this position needs to be qualified by the scale of trait expression. In evolutionary terms, alien DNA insertion occurred as an isolated, point source event, which was then selected for or against. In that context, alien gene insertion provided an opportunity for genetic change and adaptation. In contrast, tens of millions of hectares of land sown to GM crops may direct selection in novel ways. Even rare events become probable when occurring on the scale of commercial agriculture. When analyzing scaling issues in risk assessment of GMOs, Van Damme (1992) noted that it is not a question of “if” but “when.”

#### *Weediness on agricultural land*

The FSE trial reviewed earlier in the paper (Firbank, 2003) clearly showed that GM technology was more effective in controlling weeds. However, studies reviewed by Martinez-Ghersa et al. (2003) showed contradictory results and raised the additional issues of HT in target weeds and HT crop volunteers. Of the six weed species, which now have biotypes resistant to glyphosate – *Lolium rigidum* (rigid ryegrass), *Eleusine indica* (goosegrass), *Conyza canadensis* (marestail), *Lolium multiflorum* (Italian ryegrass), *Conyza bonariensis* (hairy fleabane), and *Plantago lanceolata* (buckhorn plantain) (<http://www.weedscience.org/>) – one appears to have resulted from growing RR crops. The presence of RR-marestail biotypes in soybean or cotton fields in each of 10 US states suggests that use of glyphosate on RR-crops has created or exacerbated a weed risk on agricultural land. Martinez-Ghersa et al. (2003) also referenced Roundup resistance in two other soybean weeds – common waterhemp (*Amaranthus rudis*) and velvetleaf (*Abutilon theophrastii*). Whether Roundup resistance in agricultural weeds reflects herbicide mismanagement or a predictable risk of GM technology is an arguable point.

Gulden et al. (2003) and Van Acker et al. (2003) reviewed a parallel concern with the weediness of RR-crop volunteers in agricultural land in western Canada. Volunteers are crop plants that germinate and grow in subsequent crops from seed inadvertently dropped to the ground at harvest. In a 2-year study over 35 fields and 15 different producers, Gulden et al. (2003) documented canola seed losses at harvest averaging 107 kg ha<sup>-1</sup>, or 20 times the normal seeding rate of canola. Data reviewed by Van Acker et al. (2003) showed that canola or wheat can emerge from the soil seedbank for several years after harvest, acting as HT weeds in the subsequent crops.

Because of its low cost and efficacy, Roundup has been the herbicide of choice for low-disturbance, direct seeding (e.g. conservation tillage), which is now employed on 25–30% of the cropped land in western Canada (Van Acker et al., 2003). Because Roundup is both cheap and widely used in conservation tillage, RR-crop volunteers necessitate use of herbicides costing from one to five times as much as Roundup alone. Inadvertent gene stacking resulting in seed resistant to more than one herbicide has been reported (Hall et al., 2000). Whether the cost and difficulty of addressing crop volunteers tolerant to one or more herbicides is sufficient to discourage conservation tillage – with adverse environmental implications for soil and water management in the prairies – remains an open question.

Because of the mobility of canola seed and pollen, these additional costs are imposed on non-adopters as well as adopters of the RR technology (Van Acker et al., 2003). Friesen et al. (2003) compared 33 seed lots of certified canola seed of which 18 were non-GM; 8 and 5 were tolerant to glufosinate ammonium or imidazolinone, respectively. Thirty-two of the 33 seed lots (97%) had detectable levels of adventitious contamination with one or more of the three HT traits (RR was the third), of which 14 (42%) exceeded the purity guideline for certified canola seed (0.25% same crop contamination). The RR trait contaminated 81% of the seedlots, to a maximum of 4.89%, while 21% of the seedlots were contaminated adventitiously with both RR and glufosinate ammonium resistance. Intra-specific gene flow among certified seed fields means that, whether through inadvertent seed or pollen drift from neighbors or as a contaminant in non-GM certified seed, non-adopters experience the same weed control difficulties as adopters.

Thus, widespread use of traits which are adaptive in a managed ecosystem – such as HT – creates or exacerbates weed control problems, whether by promoting

resistance in target weeds or HT crop volunteers. Inadvertent gene flow, including trait stacking, can further complicate weed control as well as broadening impact to non-adopters of the technology.

#### *Risk of invasiveness*

The CBCGEO (2004) considered invasiveness the greatest environmental risk because of its potential to alter ecosystem structure and function. Since invasiveness has been the subject of several recent reviews, evidence for gene flow to wild relatives and persistence in the wild will concentrate on more recent papers.

#### *Containability*

If genes – GM or non-GM – were fully containable, concerns about environmental impact would be limited to the point of release. However, GMOs have proven to be uncontainable, as shown by StarLink maize (Smyth et al., 2002), by the movement of GM traits into native landraces in southern Mexico (Quist & Chapela, 2001), by pollen and seed movement in Canadian canola (Friesen et al., 2003; Gulden et al., 2003; Van Acker et al., 2003), and by pharmaceutical crops.

The CBCGEO (2004) stated that not all GMOs require containment and analyzed the efficacy of available or potential genetic approaches for those that require containment. Apart from greenhouse production, the methods reviewed either had significant practical limitations (sterile triploids), or were still under development (cleistogamy or apomixis). Of the agronomic approaches reviewed by Clark (2004), including buffer zones to isolate pollen sources, harvesting outer rows separately from the rest of the field, reducing pollen synchrony through varied planting time, segregating crops on a regional basis, or installing physical barriers, including windbreaks, barren soil, or pollen trap crops, temporal staggering showed most promise but none were reliable in the field. Because absolute containment is unachievable under field crop conditions, gene flow into the environment should be assumed.

Dissemination of GM or non-GM traits can occur through seed movement as well as from intra- and interspecific outcrossing. Sexual outcrossing to related species, as between cultivated and weedy beets (*Beta* sp.) (Desplanque et al., 1999) or from sorghum (*Sorghum bicolor*) to Johnsongrass (*S. halepense*), has the potential to create crop/weed hybrids (Ellstrand et al., 1999). Ellstrand et al. (1999) found that 12 of the

13 most important crops in the world hybridize with wild relatives somewhere around the world.

Movement of adaptive genes into wild relatives has the potential to enhance weediness in a managed ecosystem or invasiveness in a natural ecosystem. For Bt, which could be adaptive in unmanaged as well as managed systems, Letourneau et al. (2002) tabulated sexually compatible wild relatives as: two for sugar beet, eight for canola, two for the vegetable brassicas (*Brassica oleracea*), seven for cotton, three for tomato (*Lycopersicon esculentum*), five for rice (*Oryza sativa*), seven for potato (*Solanum tuberosum*), and six for maize

Wang et al. (2001) examined gene flow from wheat to jointed goatgrass (*Aegilops cylindrica*). Of specific concern is the possible transfer of the RR trait from wheat (an allohexaploid consisting of the A, B, and D genomes) to jointed goatgrass (an allotetraploid of the C and D genomes). Jointed goatgrass is already a major weed in wheat-growing regions. The similarity in genetic background and growth habit of these two species, including a partial overlap in flowering intervals, increases the probability of hybridization. Wang et al. (2001) demonstrated that hybridization could indeed occur in both directions, that backcrossing could occur under natural conditions, and further, that partial self-fertility could be restored after just two backcross (BC) generations. Retention of the RR gene in jointed goatgrass, and hence, the exacerbation of an already troublesome weed, would be most likely if the RR gene is in the D genome which shows homology between the two species. However, retention on unshared genomes could also be encouraged by selection pressure for the RR trait (Wang et al., 2001). With the RR trait in the D genome, Hedge and Waines (2004) calculated that the probability of recovering BC2 seed with jointed goatgrass as the recurrent parent as one in 1.54 million plants or less, and discounted the probability of this avenue of gene flow.

Olofsdotter et al. (2000) cited evidence of the ease with which a gene for nuclear male sterility had moved from breeding plots to weedy rice in Brazil after just 2 years in the same field. They also compared seed dormancy and shattering in rice and weedy rice, which will influence the rate of spread of GM traits into weedy relatives. Olofsdotter et al. (2000) affirmed that gene movement from herbicide-tolerant rice into weedy rice will occur.

Rieseberg et al. (1999) found that the rate of naturally occurring hybridization between cultivated sunflower and its wild, sympatric relative *H. petiolaris*,

was only 0.6–2.6%, and concluded that risk of transgene escape into *H. annuus* was of more immediate concern than that into *H. petiolaris*. Faure et al. (2002) reported that the only barrier to gene flow among cultivated and wild sunflower was spatial isolation, and commented on the impossibility of predicting *a priori* risks of transgene movement into wild relatives.

Wilkinson et al. (2000) used remote sensing to identify possible sites of sympatry between GM canola and its two progenitor species, wild turnip (*Brassica rapa*) and wild cabbage (*B. oleracea*), in a 15,000 km<sup>2</sup> region of southeast England. They found only two sympatric populations with *B. rapa* and 1 with *B. oleracea*, within which they identified a single *B. rapa* hybrid (0.2% of tested plants) and none with *B. oleracea*. They concluded that the rarity of these two progenitor species limits risk of outcrossing in the region, with *B. rapa* being more probable than *B. oleracea*.

Norris and Sweet (2002) monitored gene flow between adjacent experimental fields of GM and non-GM canola at two sites in the UK. Although the potential for GM gene flow into non-GM fields was demonstrated, Norris and Sweet (2002) were unable to find evidence of natural cross-pollination between GM canola and wild radish (*Raphanus raphanistrum*), white mustard (*Sinapis alba*) or charlock (*S. arvensis*) despite extensive sampling. Hybridization was demonstrated, however, between sown plots of GM canola and turnip rape (*B. rapa*), declining from 0.25% at 1 m to 0.008% at 41 m. Extensive hybridization was also documented between canola and wild turnip, a self-incompatible species (Norris and Sweet, 2002). Thus, the potential for transgene movement from GM canola was evident with wild turnip but not with other related species, and is most likely with self-incompatible species.

Pascher and Gollmann (1999) reviewed the potential for hybridization between GM crops and wild species in Austria. Among the crosses where gene flow between sown and wild species was considered “highly probable” were carrots (*Daucus carota*), alfalfa (*Medicago sativa*), oilseed rape, forage grasses, and members of the Rosaceae, Salicaceae. According to McCreight and Staub (1999) cucurbits cross so readily with wild relatives that the flow of GM genes is probable.

Snow et al. (2001) compared the fitness of wild radish and hybrids between wild and commercial radish (*R. sativus*). Despite lower F<sub>1</sub> fitness, cultivated radish plants nonetheless persisted to account for 8–22% of the population after 3 years. They found that the F<sub>1</sub> generation did not pose a significant block to

gene transfer from cultivated to wild radish and that fitness-enhancing genes would persist.

McPherson et al. (2004) reviewed the potential for gene flow from transgenic safflower (*Carthamus tinctorius*) to weedy relatives in western Canada. They concluded that hybridization is biologically possible in selected areas of Argentina, Chile, and several states in the United States, where naturalized populations of weedy relatives exist. However, the absence of naturalized populations may make western Canada suitable for growing transgenic safflower without risk of gene flow to relatives.

#### Persistence

Persistence in an environment, whether as feral individuals or as retained GM traits within a population, is most likely if the traits are of adaptive value, as in conferring weediness/invasiveness, pest resistance, stress tolerance, lifecycle changes, tolerance to toxins, allelopathic or hormonal effectors (Warwick et al., 1999). However, as noted by Rissler and Mellon (1996), *neutral* transgenes that confer neither an advantage nor a disadvantage may also persist due to GM pollen from repeated, large-scale plantings. Transgenes can also persist in the wild through hybridization if they do not exact a metabolic penalty. Snow et al. (1999) reported that introgression of glufosinate tolerance from canola into wild mustard (*Brassica kaber*) did not affect reproductive parameters in growth room-grown plants. Due to negligible fitness costs, they concluded that the trait should be able to persist in the wild.

If GMOs were sufficiently enfeebled and could not persist without human intervention, then containability would be a non-issue. Evidence of the inability of GM or non-GM canola, potatoes, maize, and beets to persist against competition from native perennial weeds in the UK was cited by Crawley et al. (2001). Norris and Sweet (2002) provided convincing evidence that canola is ill-adapted to survival in undisturbed environments.

Yet as noted by Warwick et al. (1999) and others, crops that are not fully domesticated, such as sunflower or alfalfa, already possess weediness traits. Pascher and Gollmann (1999) reported that domesticated alfalfa frequently escaped and re-established itself, particularly in ruderal environments in Austria. Sukopp and Sukopp (1993) tabulated cultigens that have already become feral in Central Europe through hybridization with or without close relatives. Their list included various beet and brassica species, as well as foxtail millet (*Setaria italica*), carrot, alfalfa, oats (*Avena sativa*), and numerous others. They also referenced a large and

varied group of species labeled as agriophytes, namely, species which “have moved into alien regions exclusively through activities of people, but now form an integral part of the natural vegetation.”

Sukopp and Sukopp (1993) drew from population biology to show that the likelihood of a cultivar – and specifically a GM cultivar – becoming feral is directly related to the size and frequency of its deliberate release into the environment. They acknowledged the improbability of highly dependent species such as maize succeeding in the wild. However, they drew attention to the large number of other species that are less domesticated, some of which have the potential to become feral. Thus, the capacity to persist in the environment independent of human intervention can be discounted for some species, but has already been demonstrated for many others.

In nature, HT would confer no selective advantage. Insect resistance, however, could be of adaptive value in wild as well as cultivated plants. Stewart et al. (1997) demonstrated that canola plants transformed with the Bt (Cry1Ac) gene were resistant to defoliation by diamondback moth (*Plutella xylostella*) under field conditions. They discussed the implications of transferring this trait to any of nine species already known to be compatible and concluded that fixing insect resistance in species such as wild mustard would be ecologically undesirable in either managed or natural ecosystems. Using male-sterile GM sunflower, Snow et al. (2002) demonstrated the efficacy of the Bt (Cry1Ac) gene in deterring herbivory from Lepidopteran, but not weevil or fly species. Given the readiness with which commercial and native sunflower hybridize, they predicted that release of Bt sunflower would reduce herbivory and increase seed production in wild and weedy sunflowers.

Thus, while domestication has so fully altered some species as to prevent escape and persistence, other species have demonstrated the ability to revert to the wild. Many species which cannot themselves persist in unmanaged ecosystems, nonetheless have compatible relatives which can receive and retain adaptive GM traits. However, the ecological implications – if any – of transgene escape would be trait- and context-specific and are not yet available in the literature.

#### *Non-target effects*

Effects on non-target organisms have been demonstrated for a number of GMOs. How documented responses in species composition or viability could affect the integrity of larger scale ecological processes is less

clear. Reflecting the availability of published literature, discussion of non-target effects will be limited to the Bt trait, which together with HT, accounts for almost all GM hectareage. Due to space limitations, consideration will be limited to Bt maize.

The Bt protein exists in numerous Cry forms, of which several have been engineered into crops to provide selective control of particular pest species. The type of Bt (kurstaki) employed in Bt maize hybrids has, until recently, been specific to Lepidopteran species, with European cornborer (*Ostrinia nubilalis*) being a key target. The effects of Bt-maize pollen on non-target Lepidopteran species have been reported by Jesse and Obrycki (2000), Losey et al. (1999, 2002), Sears et al. (2001), Wraight et al. (2000), and others.

These studies have demonstrated that vulnerability to the endotoxin in Bt maize varies not only among Lepidopteran species but also among the various Bt insertion events. As a result, studies differ in the estimated impact of Bt maize on non-target species (NRC, 2002). For example, Sears et al. (2001) reported that the Cry1A proteins found in Bt maize were toxic to monarch (*Danaus plexippus*) butterflies, with an LD<sub>50</sub> of 3.3 ng protein ml<sup>-1</sup> diet and growth inhibition (EC<sub>50</sub>) at 0.76 ng protein ml<sup>-1</sup>. However, expression of the Cry1Ab endotoxin in pollen varied by 2 orders of magnitude between commercial Bt hybrids expressing event 176 (1.1–7.1 μg g<sup>-1</sup> pollen) and those expressing either the Mon 810 or Bt-11 events (0.09 μg g<sup>-1</sup> pollen). Despite the documented importance of maize fields for monarch habitat and oviposition sites (Oberhauser et al., 2001), toxicity and pollen density measurements under field conditions led Sears et al. (2001) to conclude that risk to monarch butterflies from pollen of either Mon 810 or Bt-11 events was negligible. Because of its greater toxicity, the limited commercial success event of 176 hybrids was fortunate for monarch butterflies, given its greater toxicity (NRC, 2002).

Monarchs are not the only Lepidopteran species potentially impacted by Bt-maize pollen. Pollen from Pioneer 34R07 (event Mon 810) had no effect on black swallowtail butterflies (*Papilio polyxenes*), while Max 454 (event 176) pollen proved to be toxic, just as it was to monarchs (Wraight et al., 2000). Identification of herbivore species at risk from Bt crops depends upon the level, phenology, and profile of toxin expression among plant tissues (Losey et al., 2002). They identified 57 plant species that exist within the pollen shadow of maize (at least 60 m and possibly 200 m), and 206 species of Lepidoptera feeding on plants within the maize pollen shadow. However, only

species with larvae feeding in the month of pollen shed, between July and mid-August, could be impacted. The monarch is particularly vulnerable because its obligate feed source – milkweed (*Asclepias* spp.) – occurs within and near maize fields, and its larvae feed during the interval of pollen shed (Losey et al., 2002).

Additional non-target effects of Bt crops were reviewed by Stotzky (2002). Evidence from Stotzky's laboratory suggests that the endotoxin released from Bt maize is not harmful to earthworms or to total culturable levels of bacteria, actinomycetes, fungi, protozoa, or nematodes. Blackwood and Buyer (2004) compared the effect of two Bt-maize hybrids, one with Cry1A (event Bt-11) and the other with Cry1F, grown in three soils, on community structure of rhizospheric microbes. Based on 36-day-old, growth chamber grown plants, they concluded that Bt effects were small and transitory.

Saxena et al. (1999) demonstrated that the roots of Bt crops exude active Bt endotoxin throughout the growing season. Stotzky (2002) cited evidence that the insecticidal activity of the Bt endotoxin persists for at least 234 days in the soil, protected from degradation by binding to clay particles. He proposed that Bt can act as a model system for studying the role of surface active particles in binding and protecting biomolecules from degradation, particularly the novel vaccines, hormones, and other bioactive compounds which may be introduced into the soil through biopharming or bio-industrial crops.

## Conclusions

At issue are contemporary approaches to environmental risk assessment for GM crops, starting with which risks are considered and which parameters are considered predictive of risk from transgenic traits. Results from targeted field-scale research, coupled with accumulating farm experience suggest opportunities for improvement. Particular attention is drawn to the need for a more holistic approach to assessment, acknowledging effects, which may ramify through trophic levels, as shown by the FSE trial in the UK. Post-release monitoring is needed to validate pre-release assessments of environmental impact.

Areas where GM crops may pose unique risks relate to the specific traits chosen for commercialization, and to the potential for unintended trait expression owing to the process of transgene insertion. Of the two traits that account for almost all of the land sown to GM crops to

date, HT has created or exacerbated weed control problems in agricultural fields but would not enhance fitness in the wild. The Bt pesticidal plant trait has not yet been found problematic in farmer's fields, but may enhance fitness in wild relatives. Third generation traits, such as those in pharmaceutical or bio-industrial crops, may present novel challenges to nature, necessitating rigorous, case-by-case analysis.

Authoritative analysts have argued that the potential for unintended trait expression is not unique to GMOs and, indeed, occurs through conventional plant breeding as well. However, both experimental and agronomic evidence documented disease, stress, and yield responses in crops bearing the RR trait. Because the RR trait acts on the shikimic acid pathway, further study is needed to separate the genetic and physiological contributions to these linkages. Nonetheless, the unintended trait effects were detected specifically because they were of research or agronomic interest, leaving unanswered the question of whether other, less visible traits may also have been affected.

Both the standard against which GM impact is to be compared and the scale of the comparison need to be considered when assessing potential for environmental risk. Several years of commercial experience in the United States and Canada have revealed weediness problems created or exacerbated by the most widely adopted GM trait – HT and specifically the RR trait – although similar problems could have occurred had a non-GM HT trait been equally cheap and effective. No practical method for full containment has yet been found, suggesting that gene flow from GM as from non-GM crops will occur to wild relatives. Retention of GM traits, either as feral plants or in wild relatives, would be most likely when the traits enhance fitness, exact no metabolic cost, or are routinely augmented by repeated, large-scale release from sown GM crops. Non-target effects of Bt maize appear to be limited to Lepidopteran species, with vulnerability varying both among species and among Bt events. Whether retained GM or non-GM traits could affect ecological or evolutionary processes awaits further study.

## References

- Al Khatib, K. & J. Miller, 2000. Registration of four genetic stocks of sunflower resistant to imidazolinone herbicide. *Crop Sci* 40: 869–870.
- Benbrook, C., 2001. Troubled times amid commercial success for Roundup Ready Soybeans. *Northwest Science* and

- Environmental Policy Center, Sandpoint, Idaho. AgBioTech Info Net Technical Paper No. 4 (3 May, 2001) ([www.biotech-info.net/troubledtimes.html](http://www.biotech-info.net/troubledtimes.html)).
- Bergelson, J., C.B. Purrington & G. Wichmann, 1998. Promiscuity in transgenic plants. *Nature* 395: 25.
- Blackwood, C.B. & J.S. Buyer, 2004. Soil microbial communities associated with Bt and non-Bt corn in three soils. *J Environ Qual* 33: 832–836.
- Brooks, D.R., et al., 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates *Philos Trans R Soc Lond Ser B* 358: 1847–1862.
- CBCGEO (Committee on Biological Confinement of Genetically Engineered Organisms), 2004. Biological confinement of genetically engineered organisms, 255 pp. National Academies Press, Washington, DC.
- CFIA (Canadian Food Inspection Agency, 2002. Decision Document DD2002-39 Determination of the Safety of Aventis CropScience Canada Inc. Glufosinate Ammonium Tolerant Sugar Beet (*Beta vulgaris*) Lines Derived from Event T120–7 (<http://www.inspection.gc.ca/english/plaveg/bio/dd/dd0239e.shtml#A9>).
- Chandler, V.L. & H. Vaucheret, 2001. Gene activation and gene silencing. *Plant Physiol* 125: 145–148.
- Clark, E.A., 2004. GM crops are not containable. In: B. Breckling & R. Verhoeven (Eds.), Risk Hazard Damage. Specification of Criteria to Assess Environmental Impact of Genetically Modified Organisms. *Naturschutz und Biologische Vielfalt. Ecological Society of Germany, Austria and Switzerland*. Hanover, Germany, 8–9 December 2003, pp. 91–108.
- Colyer, P.D., T.L. Kirkpatrick, W.D. Caldwell & P.R. Vernon, 2000. Root-knot nematode reproduction and root galling severity on related conventional and transgenic cotton cultivars. *J Cotton Sci* 4: 232–236.
- Crawley, M.J., S.L. Brown, R.S. Hails, D.D. Kohn & M. Rees, 2001. Transgenic crops in natural habitats. *Nature* 409: 682–683.
- Dale, P.J., B. Clarke & E.M.G. Fontes, 2002. Potential for the environmental impact of transgenic crops. *Nat Biotechnol* 20(6): 567–574.
- Demeke, T., P. Huci, M. Baga, K. Cawell, N. Leung & R.H. Chibbar, 1999. Transgene inheritance and silencing in hexaploid spring wheat. *Theor Appl Genet* 99: 947–953.
- De Neve, M., S. De Bock, C. De Wilde, H. Van Houdt, I. Strobbe, A. Jacobs, M. Van Montagu & A. Dipicker, 1999. Gene silencing results in instability of antibody production in transgenic plants. *Mol Gen Genet* 260: 582–592.
- Descalzo, R.C., Z.K. Punja, C.A. Levesque & J.E. Rahe, 1998. Glyphosate treatment of bean seedlings causes short-term increases in *Pythium* populations and damping off potential in soils. *Appl Soil Ecol* 8: 25–33.
- Desplanque, B., P. Boudry, K. Broomberg, P. Saumitou-Laprade, J. Cuguen & H. Van Dijk, 1999. Genetic diversity and gene flow between wild, cultivated and weedy forms of *Beta vulgaris* L. (Chenopodiaceae), assessed by RFLP and microsatellite markers. *Theor Appl Genet* 98: 1194–1201.
- Dewar, A.M., M.J. May, I.P. Woiwod, L.A. Haylock, G.T. Champion, B.H. Garner, R.J.N. Sands, Aiming Qi & J.D. Pidgeon, 2003. A novel approach to the use of genetically modified herbicide tolerant crops for environmental benefit. *Proc Royal Soc Lond B* 270: 335–340.
- Ellstrand, N.C., H.C. Prentice & J.E. Hancock, 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Ann Rev Ecol Syst* 30: 539–563.
- Elmore, R.W., F.W. Roeth, R.N. Klein, S.Z. Knezevic, A. Martin, L.A. Nelson & C.A. Shapiro, 2001a. Glyphosate-resistant soybean cultivar response to glyphosate. *Agron J* 93: 404–407.
- Elmore, R.W., F.W. Roeth, L.A. Nelson, C.A. Shapiro, R.N. Kelin, S.Z. Knezevic & A. Martin, 2001b. Glyphosate resistant soybean cultivar yields compared to sister lines. *Agron J* 93: 408–412.
- Faure, N., H. Serieys & A. Berville, 2002. Potential gene flow from cultivated sunflower to volunteer, wild *Helianthus* species in Europe. *Agric Ecosyst Environ* 89: 183–190.
- Fernandez, M.R., F. Selles, D. Gehl, R.M. DePauw & R.P. Zentner, 2003. Identification of crop production factors associated with the development of Fusarium head blight in spring wheat in southeast Saskatchewan. Manitoba Agronomists Conference, Winnipeg, MB Canada ([http://www.umanitoba.ca/afs/agronomists\\_conf/2003/pdf/fernandez.fusarium\\_hd.blight.pdf](http://www.umanitoba.ca/afs/agronomists_conf/2003/pdf/fernandez.fusarium_hd.blight.pdf)).
- Firbank, L.G., et al., 2003. An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *J Appl Ecol* 40: 2–16.
- Friesen, L.F., A.G. Nelson & R.C. Van Acker, 2003. Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agron J* 95: 1342–1347.
- Gidding, G.D., 1999. The role of modelling in risk assessment for the release of genetically engineered plants. In: K. Ammann, Y. Jacot, G. Kjellsson & V. Simonsen (Eds.), *Methods for Risk Assessment of Transgenic Plants. Ecological Risks and Prospects of Transgenic Plants*, pp. 31–41. Birkhauser Verlag, Basel.
- Gulden, R.H., S.J. Shirtliffe & A.G. Thomas, 2003. Harvest losses of canola cause large seedbank inputs. *Weed Sci* 51: 83–86.
- Hall, T.C., S.P. Kumpatla, M.B. Chandrasekharan, L.M. Iyer, G. Li & T.C. Hall, 1998. Genome intruder scanning and modulation systems and transgene silencing. *Trends Plant Sci* 3: 97–104.
- Hall, L.M., A.K.C. Topinka, J. Huffman, L. Davis & A. Good, 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Sci* 48: 688–694.
- Hanson, K.G. & M.R. Fernandez, 2003. Effect of glyphosate-based herbicides on pathogenic fungi of cereal crops. *Can J Plant Sci* 83(1): 124 (Abstract).
- Haughton, A.J., et al., 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. *Philos Trans R Soc Lond Ser B* 358: 1863–1877.
- Hawes, C., et al., 2003. Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos Trans R Soc Lond Ser B* 358: 1899–1913.
- Heard, M.S., et al., 2003. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. I. Effects on abundance and diversity. *Philos Trans R Soc Lond Ser B* 358: 1819–1832.
- Hedge, S.G. & J.G. Waines, 2004. Hybridization and introgression between bread wheat and wild and weedy relatives in North America. *Crop Sci* 44: 1145–1155.
- Inose, T. & K. Murata, 1995. Enhanced accumulation of toxic compounds in yeast cells having high glycolytic activity: A case study on the safety of genetically engineered yeast. *Int J Food Sci Technol* 30: 141–146.

- Jesse, L.C.H. & J.J. Obrycki, 2000. Field deposition of Bt transgenic corn pollen: Lethal effects on the monarch butterfly. *Oecologia* 125: 241.
- King, C., L. Purcell & E. Vories, 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybeans in response to foliar application. *Agron J* 93: 179–186.
- Kumpatla, S.P., M.B. Chandrasekharan, L.M. Iyer, G. Li & T.C. Hall, 1998. Genome intruder scanning and modulation systems and transgene silencing. *Trends Plant Sci* 3: 97–104.
- Lee, C.D., D. Penner & R. Hammerschmidt, 2003. Glyphosate and shade effects on glyphosate-resistant soybean defense responses to *Sclerotinia sclerotiorum*. *Weed Sci* 51: 294–298.
- Letourneau, D.K. & B.E. Burrows, 2002. Genetically Engineered Organisms. Assessing Environmental and Human Health Effects. CRC Press, Boca Raton.
- Letourneau, D.K., J.A. Hagen & G.S. Robinson, 2002. Bt-crops: Evaluating benefits under cultivation and risks from escaped transgenes in the wild. In: D.K. Letourneau & B.E. Burrows (Eds.), *Genetically Engineered Organisms. Assessing Environmental and Human Health Effects*, pp. 33–98, Chapter 3. CRC Press, Boca Raton.
- Losey, J.E., L.S. Rayor & M.E. Carter, 1999. Transgenic pollen harms monarch larvae. *Nature* 399: 214.
- Losey, J.E., J.J. Obrycki & R.A. Hufbauer, 2002. Impacts of genetically engineered crops on non-target herbivores: Bt-corn and monarch butterflies as a case study. In: D.K. Letourneau & B.E. Burrows (Eds.), *Genetically Engineered Organisms. Assessing Environmental and Human Health Effects*, pp. 143–165, Chapter 6. CRC Press, Boca Raton.
- Mack, R.N., D. Simberloff, W.M. Lonsdale, H. Evans, M. Clout & F.A. Bazzaz, 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecol Appl* 10: 689–710.
- Martinez-Ghersa, M.A., C.A. Worster & S.R. Radosevich, 2003. Concerns a weed scientist might have about herbicide-tolerant crops: A revisit. *Weed Technol* 17: 202–210.
- Marvier, M.A., E. Meir & P.M. Kareiva, 1999. How do the design of monitoring and control strategies affect the chance of detecting and containing transgenic weeds? In: K. Ammann, Y. Jacot, G. Kjellsson & V. Simonsen (Eds.), *Methods for Risk Assessment of Transgenic Plants. III. Ecological Risks and Prospects of Transgenic Plants*. Birkhauser Verlag, Basel.
- Marvier, M., 2002. Improving risk assessment for nontarget safety of transgenic crops. *Ecol Appl* 12: 1119–1124.
- McCreight, J.D. & J. Staub, 1999. Report of the cucurbit working group. In: P.L. Traynor & J.H. Westwood (Eds.), *Ecological Effects of Pest Resistance Genes in Managed Ecosystems*, pp. 79–87. Information Systems for Biotechnology, Blacksburg, VA.
- McPherson, M.A., A.G. Good, A.K.C. Topinka & L.M. Hall, 2004. Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World. *Can J Plant Sci* 84: 923–934.
- Mellon, M. & J. Rissler, 2003. Environmental Effects of Genetically Modified Food Crops. Recent Experiences. Union of Concerned Scientists August Update ([http://www.ucsusa.org/food\\_and\\_environment/biotechnology/page.cfm?pageID=1219](http://www.ucsusa.org/food_and_environment/biotechnology/page.cfm?pageID=1219)).
- Meyer, 1996. Inactivation of gene expression in transgenic plants. In: J. Tomiuk, K. Wohrmann & A. Sentker (Eds.), *Transgenic Organisms: Biological and Social Implications*. Birkhauser Verlag, Basel.
- NRC (National Research Council), 2002. *Environmental Effects of Transgenic Plants*, 320 pp. National Academy Press, Washington, DC.
- Nelson, K.A., K.A. Renner & R. Hammerschmidt, 2002. Cultivar and herbicide selection affects soybean development and the incidence of *Sclerotinia* stem rot. *Agron J* 94: 1270–1281.
- Njiti, V.N., O. Meyers Jr., D. Schroeder & D.A. Lightfoot, 2003. Roundup ready soybean: Glyphosate effects on *Fusarium solani* root colonization and sudden death syndrome. *Agron J* 95: 1140–1145.
- Norris, C. & J. Sweet, 2002. Monitoring Large Scale Releases of Genetically Modified Crops (EPG 1/5/84) (<http://www.defra.gov.uk/environment/gm/research/epg-1-5-84.htm>).
- Oberhauser, K.S., M. Prysby, H.R. Mattila, D.E. Stanley-Horn, M.K. Sears, G. Dively, E. Olson, J.M. Pleasants, W.K.F. Lam & R.I. Hellmich, 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proc Natl Acad Sci USA* 98: 11913–11918.
- Olofsdotter, M., B.E. Valverde & K.H. Madsen, 2000. Herbicide resistant rice (*Oryza sativa* L.): Global implications for weed rice and weed management. *Ann Appl Biol* 136: 279–294.
- Pascher, K. & G. Gollmann, 1999. Ecological risk assessment of transgenic plant releases: An Austrian perspective. *Biodiv Conserv* 8: 1139–1158.
- Pew Initiative on Food and Biotechnology, 2001. *Harvest on the Horizon: Future Uses of Agricultural Biotechnology*. Pew Initiative on Food and Biotechnology, University of Richmond, Virginia.
- Pline, W.A., R. Wells, G. Little, K.L. Edmisten & J.W. Wilcut, 2003. Glyphosate and water-stress effects on fruiting and carbohydrates in glyphosate-resistant cotton. *Crop Sci* 43: 879–885.
- Purrlington, C.B. & J. Bergelson, 1995. Assessing weediness of transgenic crops: Industry plays plant ecologist. *Tree* 10: 340–342.
- Quist, D. & I.H. Chapela, 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414: 541–543.
- Reddy, A.S. & T.L. Thomas, 1996. Expression of a cyanobacterial  $\delta^6$ -desaturase gene results in  $\gamma$ -linolenic acid production in transgenic plants. *Nat Biotechnol* 14: 639–642.
- Reddy, K.N. & R.M. Zablotowicz, 2003. Glyphosate-resistant soybean response to various salts of glyphosate and glyphosate accumulation in soybean nodules. *Weed Sci* 51: 496–502.
- Rieseberg, L.H., M.J. Kim & G.J. Seiler, 1999. Introgression between the cultivated sunflower and a sympatric wild relative, *Helianthus petiolaris* (Asteraceae). *Int J Plant Sci* 160: 102–108.
- Rissler, J. & M. Mellon, 1996. *The Ecological Risks of Engineered Organisms*. MIT Press, Cambridge, MA.
- Sanogo, S., X.B. Yang & H. Scherm, 2000. Effects of herbicides on *Fusarium solani* f. sp. glycines and development of sudden death syndrome in glyphosate-tolerant soybean. *Phytopathology* 90: 57–66.
- Saxena, D. & G. Stotzky, 2001. Bt corn has a higher lignin content than non-Bt corn. *Am J Bot* 88: 1704–1706.
- Saxena, D., S. Flores & G. Stotzky, 1999. Insecticidal toxin in root exudates from Bt corn. *Nature* 402: 480.
- Scientists Working Group on Biosafety, 1998. *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms. Part I. Introductory Materials and Supporting Text for Flowcharts*. The Edmonds Institute, Washington, DC.
- Sears, M.K., R.L. Hellmich, D.E. Stanley-Horn, K.S. Oberhauser, J.M. Pleasants, H.R. Mattila, B.D. Siegfried & G.P. Dively, 2001. Impact of Bt corn pollen on monarch butterfly populations:

- A risk assessment. *Proc Natl Acad Sci USA* 98(21): 11937–11942.
- Smyth, S., G.G. Khachatourians & P.W.B. Phillips. 2002. Liabilities and economics of transgenic crops. *Nat Biotechnol* 20: 537–541.
- Snow, A.A., B. Andersen & R.B. Jorgensen, 1999. Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. *Mol Ecol* 8: 605–615.
- Snow, A.A., K.L. Uthus & T.M. Culley, 2001. Fitness of hybrids between cultivated radish and weedy *Raphanus raphanistrum*: Implications for rapid evolution in weeds. *Ecol Appl* 11: 934–943.
- Snow, A.A., D. Pilon, L.H. Rieseberg, M. Paulsen, N. Pleskac, M. Reagon & D. Wolfe, 2002. A Bt transgene reduces herbivory and enhances fecundity in field populations of BC1 common sunflower (*Helianthus annuus*). In: 42nd Annual Meeting of the Weed Science Society, America, Reno, NV.
- Squire, G.R., et al., 2003. On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos Trans R Soc Lond Ser B* 358: 1779–1799.
- Stewart, C.N., J.N. All, P.L. Raymer & S. Ramachandran, 1997. Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. *Mol Ecol* 6: 773–779.
- Stotzky, G., 2002. Release, persistence, and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis*. In: D.K. Letourneau & B.E. Burrows (Eds.), *Genetically Engineered Organisms. Assessing Environmental and Human Health Effects*, pp. 187–222, Chapter 8. CRC Press, Boca Raton.
- Sukopp, H. & U. Sukopp, 1993. Ecological long-term effects of cultivars becoming feral and of naturalization of non-native species. *Experientia* 49: 210–218.
- Traynor, P.L. & J.H. Westwood, 1999. *Ecological Effects of Pest Resistance Genes in Managed Ecosystems*. Information Systems for Biotechnology. Blacksburg, VA.
- Van Acker, R.C., A.L. Brule-Babel & L.F. Friesen, 2003. *An Environmental Safety Assessment of Roundup Ready Wheat: Risks for Direct Seeding Systems in Western Canada*. Report for the Canadian Wheat Board, Winnipeg, MB (<http://www.worc.org/pdfs/WheatCWBEEnviroReportJune2003.pdf>).
- Van Damme, J.M.M., 1992. Hybridisation between wild and transgenic plants. In: J. Weverling & P. Schenkelaars (Eds.), *Ecological Effects of Genetically Modified Organisms*, pp. 81–91. Netherlands Ecological Society, Amsterdam.
- Warwick, S.I., H.J. Beckie & E. Small, 1999. Transgenic crops: New weed problems for Canada? *Phytoprotection* 80: 71–84.
- Watrud, L.S., E.H. Lee, A. Fairbrother, C. Burdick, J.R. Reichman, M. Bollman, M. Storm, G. King & P.K. Van de Water, 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. *Proc Natl Acad Sci USA* 101: 14533–14538.
- Wilkinson, M.J., I.J. Davenport, Y.M. Charters, A.E. Jones, J. Al-lainguillaume, H.T. Butler, D.C. Mason & F. Raybould, 2000. A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Mol Ecol* 9: 983–991.
- Williamson, M., 1993. Invaders, weeds and the risk from genetically manipulated organisms. *Experientia* 49: 219–224.
- Wolfenbarger, L.L. & P.R. Phifer, 2000. The ecological risks and benefits of genetically engineered plants. *Science* 290: 2088–2093.
- Wraight, C.L., A.R. Zangerl, M.J. Carroll & M.R. Berenbaum, 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proc Natl Acad Sci USA* 97: 7700–7703.
- Wang, Z., R.S. Zemetra, J. Hansen & C.A. Mallory-Smith, 2001. The fertility of wheat × jointed goatgrass hybrid and its backcross progenies. *Weed Sci* 49: 340–345.