

REVIEW

Herbicide-resistant genetically-modified crop: its risks with an emphasis on gene flow

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Herbicide-resistant genetically-modified (GM) crops are the most widely cultivated worldwide, representing 78% of GM crops in 1999, followed by insect-resistant GM crops with *Bt* gene. Gene flow is the most touching risk arising from GM crops, and is categorized as three types: within species, between species and between GM crop and other organisms. This review shows that gene flow is a reality in the plant kingdom with evolutionary change. Herbicide resistance evolves naturally and spreads dynamically in weeds. One of the most concerning crop in relation to gene flow is *Brassica napus*, which has a high outcrossing rate and many relative species. In contrast, frequency of gene flow via outcrossing is relatively low in inbreeding cereal crops such as rice, wheat and barley, but published reports have shown that substantial gene flow is possible. Another possible and immediate risk is herbicide-resistant GM crops becoming volunteer weeds. Dry direct-seeded rice is one of the most likely crops in this respect. Stacking different resistance genes in a crop would accelerate multiple resistance evolution in weeds. Multiple resistance to three major herbicides has already been observed in oilseed rape cultivation. More efforts must be made for long-term risk assessment on GM crops in the natural ecosystem. More studies on weed biology and ecology, particularly reproductive processes in weeds, are essential for better understanding of gene flow and systematic management strategy. We hope that this review motivates researchers to analyze data available now, to collect fundamental information on crops and weeds in agro-ecosystem, and to lead to better risk assessment and management.

Keywords: *Brassica napus*, gene flow, GM crop, herbicide resistance, risk assessment

INTRODUCTION

Concern is increasing over the risk of environmental contamination by genetically modified (GM) crops. Under the selection pressure of herbicide, introgression between the GM crop and wild relative could produce new biota that might disrupt ecological balances (e.g. Dale *et al.* 1993). If the transgene causes the crop to become weedy, the GM crop itself could become an environmental hazard. Although there have been many debates between scientists, governmental authorities, agricultural producers, industries and consumer groups,

any outputs from those debates have not yet given a confident acceptance of GM crops in our environmental context, particularly agro-ecosystem.

Novel traits being introduced into crop plants can be classified into three main categories: herbicide resistance, pathogen/pest resistance and food improvement parameters such as shelf-life, and the amount/composition of fatty acid, protein, vitamin or other nutritional substances (Beck & Ulrich 1993; Goy & Duesing 1995; Rogers & Parkes 1995). So far, herbicide resistance is the most common trait being tested and thus herbicide-resistant GM crops are now the most widely cultivated worldwide, reflecting both its experimental and commercial applications. Statistics show that 39.9 million ha of GM crops were cultivated worldwide in 1999. Herbicide-resistant GM crops, including insect/herbicide-resistant GM crops, represented 78%

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Table 1. Major genetically-modified (GM) crops planted in 1999

Crop	Global area of GM crops (million ha)	% GM
Herbicide-resistant soybean	21.6	54
Bt maize	7.5	19
Herbicide-resistant oilseed rape	3.5	9
Bt/herbicide-resistant maize	2.1	5
Herbicide-resistant cotton	1.6	4
Herbicide-resistant maize	1.5	4
Bt cotton	1.3	3
Bt/herbicide-resistant cotton	0.8	2
Total	39.9	100

Source: James 2000. Bt, *Bacillus thuringiensis*.

of the GM crops in this area in 1999, as shown in Table 1 (James 2000). Among herbicide-resistant GM crops, herbicide-resistant soybean was the most popular, grown in about 21.6 million ha in 1999, followed by herbicide-resistant oilseed rape (OSR) of 3.5 million ha.

Regarding countries cultivating commercialized GM crops, the number of countries increased from six in 1996 to 12 in 1999 with USA ranked first place with 28.7 million ha, followed by Argentina of 6.7 million ha and Canada of 4.0 million ha (James 2000). Interestingly, China was ranked in the fourth place with 0.3 million ha. In China from 1997 to 1999, 121 GM crop applications were approved for environmental release, field testing or commercialization, of which cotton and rice represented 33% and 26% respectively of approvals. By trait, insect and virus resistances represented 52% and 21% of GM traits, respectively, while herbicide resistance represented only 2%, indicating that there is a lower demand for herbicide resistance traits due to low agricultural labor costs for weed control in China. Chen (1999) predicted that within the next 10 years, about 20–50% of five of China's principal crops (rice, wheat, maize, soybean and cotton), grown on a total of 98.9 million ha, could be planted as GM crops, equivalent to a half of the global GM crop area in 1999 at 20% adoption rate for GM crop (James 2000).

Since 1992, herbicide resistance traits have been dominant over other traits in the application stage and approved in the USA. Statistics revealed that 44 approvals of 'non-regulated status' for GM crops were given to 19 companies in the USA from 1992 to 1999, of which herbicide-resistant GM crops were about

half (USDA-APHIS-PPQ-PRA Biotechnology Authorizations 2000). Among herbicide-resistant GM crops, glyphosate-resistant GM crops (so-called 'Roundup-ready' crops) are now most widely grown worldwide. The fact that GM crop cultivation has increased very fast in the last 5 years indicates that the consequences of the commercial release of GM crops will be realized whether they are better or worse than our prediction. Our concern arising from this point is that transgenes, particularly those responsible for herbicide resistance, may escape from the source plant to other species. Therefore, this review will focus on the risks of potential gene flow from GM crops, particularly herbicide-resistant crops, to their vicinal agro-ecosystems.

RISK CLASSIFICATION OF GM CROPS

GM crops are a reality but this has been accompanied by controversy regarding the large-scale commercial release of them both in the public (e.g. Renouf 1997) and scientific (e.g. Mikkelsen *et al.* 1996a) domains. Rieger *et al.* (1999b) classified the risks associated with GM crops into four categories: (i) the toxicity or allergenic effects of transgenes on humans, animals or beneficial organisms (e.g. Crawley *et al.* 1993); (ii) the stability of transgenes (e.g. Rogers & Parkes 1995); (iii) gene flow within and between species, and to other organisms (e.g. Jorgensen *et al.* 1996); and (iv) GM crops themselves becoming weedy (e.g. Raybould & Gray 1994). The above risks are direct impacts of GM crops, while there are also some indirect impacts of GM crops.

Drift of non-selective herbicide out of the GM crop field could cause significant damage to neighboring non-GM crops and endanger some wild plants growing nearby non-cropping area. This problem of non-selective herbicide drift could be serious, particularly in the area of diversified small-scale farming. Expanding of GM crop cultivation may also bring about the risk of increased public cost of managing herbicide-resistant weeds at roadsides and parks and other areas. Unwanted gene pollution from a GM crop to nearby non-GM crops, by genes such as antibiotic-resistance genes or *Bacillus thuringiensis* (*Bt*) toxin gene, could create conflict between the farmer who does not want GM crops and the farmer planting GM crops or the company selling GM seeds. Use of the so-called 'terminator gene' (Jury 1998) would bring about a monopolization of a market by a company that sells GM crop seeds and chemicals. These may be considered as future social risks.

Insect-tolerant GM crops express δ -endotoxin genes derived from *Bacillus thuringiensis*. The GM crop produces δ -endotoxin (Cry protein) in every cell of the plant throughout the growing season and leaves the residues in the soil.

Although the Bt toxins in the environment are biodegradable and safe (Bauer 1995; Bets *et al.* 2000), the continued and elevated presence of Bt toxins is of concern. Thomas and Ellar (1983) found that native δ -endotoxin crystals of BtI had no detectable toxicity in *in vitro* and *in vivo* systems tested, but alkali-solubilized crystal caused hemolysis of rat, mouse, sheep, horse and human erythrocytes. Intravenous or subcutaneous administration of the alkali-soluble δ -endotoxin to mice at a dose rate of 15–30 $\mu\text{g/g}$ body weight resulted in rapid paralysis followed by death. The crystalline toxins exist as protoxins which become solubilized in the alkaline gut contents of dipteran, lepidopteran and coleopteran insect larvae and undergo proteolytic cleavage to form active toxins, but would be degraded in the human stomach as they are not acid stable (Drobniewski 1994). While Bt caused in rare incidences wound and eye infection to human and bovine mastitis (Turnbull & Kramer 1999), Green *et al.* (1990) reported results of an epidemiological study conducted in an area of Oregon aerially sprayed for two successive growing seasons with *Bacillus thuringiensis* var. *kurstaki*. The human population within the area was 80 000 and 55 *Bacillus thuringiensis* var. *kurstaki* isolates were found in human body-samples taken from various sites. Of these, 52 were judged to be contaminants, but it was argued that in three cases the organism could have been the pathogen responsible for infection. *Bacillus* species are numerous and widely distributed in nature, particularly in soil. Among them 12 species have caused human disease. *Bacillus cereus*, which quite often causes food poisoning, *Bacillus anthracis*, which causes anthrax to warm-blooded animals, and *Bacillus thuringiensis* are very closely related in taxonomy and share in common many biochemical characteristics. A dry form of anthrax spores is a conventional biological weapon (Drobniewski 1994; Turnbull & Kramer 1999). Thus, the potential risk to humans of *Bacillus thuringiensis* used for biological controls and Bt toxins produced by GM crops cannot be completely eliminated.

Increased Bt toxin levels in the environment constitutes a high selection pressure for the development of Bt-toxin resistant insects. Another concern about GM crops expressing Bt toxins is that suboptimal production of toxins might result in an increased risk of pests developing Bt resistance (Daniell 1999). Hence, a

strategy called 'refuge' has been put forward recently by companies to delay or prevent appearance of insects resistant to Bt toxin. Refuge involves setting aside blocks of non-GM crops planted among the large acreage of Bt toxin-containing crops. The strategy assumes insect resistance to Bt toxin is genetically recessive. When the refuge is present, insects surviving Bt exposure do not need to hunt for rare Bt survivors for sex; they can limp over to the refuge and mate with insects bearing dominant genes. The offspring of such mating will all be sensitive to Bt toxin, but will bear recessive genes for Bt tolerance. The refuge delays the appearance of Bt-resistant insect populations (Cummins 1998). However, dominant inheritance of Bt resistance has been observed in Colorado potato beetle (Rahardja & Whalon 1995). That pest is a scourge of many vegetable crops (Cummins 1998). Also, a recent study of a resistant strain of pink boll worm larvae on Bt cotton shows developmental asynchrony, which favors assortative mating among resistant moths emerging from Bt crops, and generates a disproportionately high number of homozygous resistant insects, accelerating the evolution of Bt resistance (Liu *et al.* 1999).

Agricultural crops support not only pest insect but also beneficial insects which feed on these herbivores and which play an important role in the regulation of pest population (van Driesche & Bellows Jr 1996). Pest-resistant GM crops can thus affect natural enemies by severely depleting their supply of prey or hosts. The δ -endotoxin can kill pest insects but also non-target insects such as monarch butterflies (Losey *et al.* 1999), and beneficial invertebrates which are natural enemies of pests. Cowgill *et al.* (1999) suggested that aphids feeding on nematode-tolerant GM crops may damage various natural enemies of aphids. Nematode growth inhibitors (proteinase inhibitors) expressed in the GM crops can kill aphids and some natural enemies of aphids. Decreased populations of aphids also will adversely affect the predators of aphids. When highly tolerant crops are grown on a large scale, the abundance of some natural enemies may also decline due to prey depletion (Schuler *et al.* 1999).

Consumer groups are very concerned about the safety of GM foods because they know where the transgene came from. Most gene sources are microorganisms such as *Bacillus* and *Agrobacterium* species (Table 2). Although humans have evolved in the same environment as these microorganisms, we have never dreamed of eating food containing their products regardless of their harmfulness.

Introducing a new gene may break a genetic balance in the natural population, causing more vigorous gene

Table 2. Petitions for non-regulated status of GM crops approved in the USA from 1992 to 1999

Trait	Gene	Donor	Crop
Herbicide resistance to			
Bromoxynil	Nitrilase	<i>Klebsiella pneumoniae</i>	Maize
Glyphosate	EPSPS	Maize, <i>Agrobacterium</i> sp.	Maize, oilseed rape, soybean, beet
Phosphinothricin	Phosphinothricin, acetyl transferase	<i>Streptomyces viridochromogenes</i> , <i>Agrobacterium tumefaciens</i> , <i>Streptomyces hygroscopicus</i>	Cotton, maize, beet, oilseed rape, rice, soybean
Sulfonylurea	Acetolactate synthase	Tobacco	Cotton
Soil residues of sulfonylurea			Flax
Insect resistance to			
Coleopteran	CryIIIA	<i>Bt</i>	Potato
Colorado potato beetle	CryIIIA	<i>Bt</i>	Potato
European corn borer	CryIA (b), CryIA (c)	<i>Btk</i>	Maize
Lepidopteran	CryIA (b), CryIA (c)	<i>Btk</i>	Cotton, maize, tomato
Herbicide/Insect resistance to			
Glyphosate/European corn borer	EPSPS Glyphosate oxidoreductase/ CryIA (b)	<i>Agrobacterium</i> sp. <i>Achromobacter</i> sp., <i>Btk</i>	Maize
Bromoxynil/lepidopteran	Nitrilase/CryIA (c)	<i>Klebsiella pneumoniae</i> / <i>Btk</i>	Cotton
Phosphinothricin/male sterility			Maize, oilseed rape
Phosphinothricin/Lepidopteran			Maize
Virus resistance to			
CMV, WMV2, ZYMV	Coat protein	CMV, WMV2	Squash
PRSV	Coat protein	PRSV	Papaya
WMV2, ZYMV	Coat protein	WMV2, ZYMV	Squash
Product quality improvement			
Oil profile	ACP thioesterase, Delta-12 desaturase	California bay, soybean	Oilseed rape, soybean
Fruit polygalacturonase level	Polygalacturonase, polygalacturonase antisense	Tomato	Tomato
Fruit ripening	S-adenosylmethionine transferase, ACC deaminase, ACC synthase, polygalacturonase antisense	Bacteriophage T3, tomato, <i>Pseudomonas chlororaphis</i>	Tomato

Source: USDA-APHIS-PPQ-PRA Biotechnology Authorizations (as of 6/30/2000). Bt, *Bacillus thurengiensis*.

flow until genetic balance is achieved. The change in the genetic balance is a kind of evolution, but the problem is that this evolutionary change by the release of GM crops is quite artificial and we have no idea of the future consequences. The consequence of gene flow is not well known, but it would be either significant or negligible depending on crop species. Among the risks classified above, thus, our concerns are gene flow from GM crops to others and the possibility of GM crops becoming volunteer weeds, in particular, GM herbicide-resistant crops.

RISKS OF GENE FLOW OUT OF GM CROPS

Gene flow within and between populations has an important role in maintaining population genetic structures, enabling adaptation to changing environmental circumstances, and reducing vulnerability to evolutionary hazards such as inbreeding depression and genetic drift (Campbell 1991; Ellstrand 1992). However, when we consider GM crops, gene flow is a different story. Escaped genes from GM crops will result in unknown consequences, as most transgenes are transferred to the crops from other organisms. Gene flow is the movement of genes mediated by pollen flow and seed dispersal (e.g. Waser & Price 1989). Potential gene flow from GM crops is the movement of seed and pollen as a function of distance (Raybould & Clarke 1999), while actual gene flow is the amount of fertilization in the case of pollen and establishment of reproductive individuals in the case of seeds as a function of distance from a source (Levin & Kerster 1974). These two processes (movement of pollen and seed, and fertilization and establishment, respectively) combine to move genes in space and time (Waser & Price 1989; Campbell 1991). Gene flow can occur within species (GM crops to the same crop species), between species (GM crops to different plant species), and from GM crops to other organisms such as microorganisms.

Mechanisms of gene flow (potential to actual)

Gene flow between GM crops and related species demands some basic conditions (Jorgensen 1999); GM crops and recipient plants must have overlapping flowering periods and the distance between the crop and recipient must allow transfer of pollen between them. After that, there must be genetic cross compatibility so that the hetero-specific pollen can germinate and fertilize the egg cell; subsequently, a hybrid embryo must develop from the germinating seed and produce a viable plant. Rieger *et al.* (1999a) summarized fundamental processes of the movement and persistence of

genes within and between plants: interspecific gene movement by pollen and seed, intraspecific gene flow via hybridization, and introgression of genes into a plant species. Gene flows in the form of pollen are mediated by pollinators or passive forces such as wind, and gene flow in the form of seeds is mediated by agronomic processes or by passive forces including wind and water (Rieger *et al.* 1999a). Hybridization between species plays a key role in gene flow across species barriers (Darmency 1994; Ellstrand *et al.* 1996). After hybridization or crosses between plants, introgression is essential for a stable incorporation of genes.

Factors related to pollen and seed movement

Distance is the most important factor of spatial gene flow in the form of both pollen and seed. Levels of cross-pollination tend to decrease with increasing distance from the pollen source. Table 3 shows that the isolation distance recommended for commercial seed production varies with crop species. For example, sunflower is recommended to be isolated by 800 m, and OSR by 40 m. When distance of pollen movement was measured, it was recorded at a distance of up to 400 m from a large release of herbicide-resistant GM OSR using male sterile bait plants (Simpson *et al.* 1999), indicating that pollen can be dispersed over considerable distance. Although airborne pollen was recorded at all sites, the density declined rapidly with distance from the source (Thompson *et al.* 1999).

Pollen movement is also determined by the fragmentation, shape and boundary of a field. Interactions both between and within fragments are important (Husband & Barrett 1996). Squire *et al.* (1999) highlighted that the distribution of fields and fragmentation of habitat in agricultural landscapes have a dominant effect on the rate of gene flow among crops and feral or wild relatives. Their result indicates that large pollen sources, such as crop fields, interacted on a regional scale to increase gene flow. Gene flow within moderate or large fragments was mainly between individuals in the fragment, while gene flow between fragments was greatest from small to large fragments (White & Powell 1997). More generally, the relative extent and direction of gene flow between and within fragments might depend on the distance between fragments, the flight behavior of insect vectors or aspects of the airborne pollen profile, as well as sexual compatibility. Mediators such as pollinators, wind and water thus have to be taken into account.

Seeds containing transgenes are categorized to two forms: (i) the seed of a GM crop which has escaped and

Table 3. Gene outflow potential of some important crops

Crop	Chromosome no. (2n) and genome	Outcross (%)	Isolation standard (m)	Compatibility with crops and relatives
Normally outcrossing crops				
Carrot	18			<i>Daucus capillifolius</i> , <i>Daucus carota</i>
Maize	20		200	Teosinte, <i>Zea mexicana</i>
Radish	18	>85	1000	<i>Brassica oleracea</i> , <i>Brassica napa</i> , <i>Sinapis arvensis</i> , <i>Raphanus raphanistrum</i> , <i>Raphanus maritimus</i> , <i>Raphanus landra</i>
Rye	14		200	Wheat, <i>Secale ancestrale</i> , <i>Secale dighoricum</i> , <i>Secale segetale</i> , <i>Secale afghanicum</i> , <i>Secale montanum</i>
Sorghum	20	30–40	300–400	Wild sorghums, <i>Sorghum halepense</i> , <i>Sorghum propinquum</i> , <i>Sorghum bicolor</i> ssp. <i>verticilliflorum</i>
Squash				Close wild or feral relatives, <i>Cucurbita lundelliana</i> , <i>Cucurbita martinenzii</i> , non-weedy congeners
Sugarbeet	18			All forms of the section <i>Beta</i>
Sunflower	34	20–75	800	<i>Helianthus tuberosus</i> , <i>Helianthus petiolaris</i> , <i>Helianthus agrophyllus</i> , <i>Helianthus agrophyllus</i>
Normally inbreeding crops				
Barley	14 (28)	Variable (max: 10)	0	Wild forms, <i>Hordeum spontaneum</i> , <i>Hordeum bulbosum</i>
Cotton	52 AADD	5–40	400	Wild congeners, <i>Gossypium arboreum</i> , <i>Gossypium herbaceum</i>
Flax	30	3	0	<i>Linum africanum</i> , <i>Linum angustifolium</i>
Lettuce	18	1–6	10	<i>Latuca sativa</i> , <i>Latuca indica</i> (<i>brevirostris</i>), <i>Latuca serriola</i> (prickly lettuce), <i>Latuca virosa</i> , <i>Latuca saligna</i>
Oat	42	0.5–1 (max: 10)	0	<i>Avena</i> spp., <i>Avena fatua</i>
Oilseed rape	38 AACC	>10	40	<i>Brassica napus</i> , <i>Brassica campestris</i> (= <i>rapa</i>), <i>Brassica nigra</i> , <i>Brassica napella</i> , <i>Brassica borugeaui</i> , <i>Brassica cretica</i> , <i>Brassica montana</i>
Potato	48 AAA'A ¹			<i>Solanum demissu</i> , <i>Solanum edinense</i> , <i>Solanum semidemissum</i> , <i>Solanum curtilobum</i> , <i>Solanum sucrense</i> , <i>Solanum andigena</i> , relatives in the subsection potato
Rice	24	1–2	3	Weedy rice and relatives, <i>Oryza rufipogon</i> , <i>Oryza nivara</i> , <i>Oryza glaberrima</i> , <i>Oryza breviculata</i>
Soybean	40	<1	0	<i>Glycine soya</i>
Tomato	24	<2	30	<i>Lycopersicon</i> spp., tomato-like <i>Solanum</i> spp.
Wheat	42 AABBDD	Variable (max: 10)	0	Tetraploids, <i>Aegilops squarrosa</i> , <i>Aegilops cylindracea</i> , <i>Secale</i> spp., <i>Hordeum</i> spp., <i>Aegilops</i> spp.

Compiled from various sources: Frankel & Galun (1977), Keeler *et al.* (1996), Kwon *et al.* (1972), Matsuo (1989), Smartt & Simmonds (1995), Won (1999).

become a volunteer weed and (ii) the seed of plants resulting from a hybridization by pollen flow between a GM crop and another plant. Their dispersal is identical, and can be divided into spatial and temporal dispersal. Before seed dispersal, seed loss is an important input factor determining the size of the potential pool of seeds subjected to dispersal. These characteristics depend on crop and weed species having different amounts of seed production and shattering habits. Spatial seed dispersal is a distance-related consequence of seed movement by agronomic processes such as combining, cultivation and transportation, and passive forces such as wind and water or active forces such as the actions of animals. Marshall & Brain (1999) modeled horizontal seed movement in arable soil by soil-cultivation. Temporal dispersal is related to seed dormancy, longevity in soil and germination ability. Genetically-modified crop seeds stacked in the soil seedbank could disperse by temporal and spatial dynamics. Thus, the temporal and spatial dispersal of GM crop seed could cause a long-lasting weed problem.

Factors related to hybridization and introgression

Genes can move between species by the process of hybridization, but there are some barriers to be overcome. For example, *Brassica napus* is known to have a high outcrossing rate of about 30% (Williams *et al.* 1986; Rakow & Woods 1987), suggesting the potential for outcross with its relative species. As shown in Table 3, each crop has a different outcross rate with other plants. Among outcrossing crops, radish has an outcross rate of 85%, and sunflower and sorghum have rates of 20–75% and 30–40% respectively. For inbreeding crops, cotton has outcross rates of 5–40%, while rice has rates of 1–2%. For the potential outcross between species to become real phenomenon, flowering time needs to overlap. And then, pollen has to maintain its viability during travel by wind or pollinators as the spatial dispersal of pollen is dependent on distance. Even if pollen lands safely on a stigma of another species, there exist barriers such as pollen compatibility and germinability. A few studies on pollen compatibility and germination have concluded that reproductive barriers are present between species (Frankel & Galun 1977).

Introgression can be defined as the stable incorporation of genes from one gene pool into another (Anderson 1949). Like hybridization, there are barriers including incompatibility, genetic instability and limited hybrid pollen fertility. Among the barriers, pollen fertility is the most critical factor for hybrid persistence (Rieger *et al.* 1999b). Pollen fertility depends on parent and receptor plant species. High pollen fertility was observed with

Brassica rapa–*B. napus* hybrids under field conditions (Jorgensen & Anderson 1994; Mikkelsen *et al.* 1996b). Kerlan *et al.* (1993) also observed high pollen fertility for hybrids from the *B. napus*–*Brassica oleraceae* combination (94%). Meiotic behavior of this hybrid was also normal (Kerlan *et al.* 1993). Thus, the production of fertile hybrids implies that transgenes with herbicide resistance may spread from a confined field of GM crops. Although transgenes that escape to other species will pose no immediate problem if selection pressure for the transgene, such as herbicide treatment, does not exist (Sindel 1997), a transgene can persist in the wild without selection pressure as shown in a recent study by Lavigne *et al.* (1995) on the fitness of a sulfonylurea resistance line of white chicory.

Risks of gene flow within species

The most possible gene flow can occur between GM crops and non-GM crops or their wild species. Within each crop species, there are hundreds of different cultivars bred using conventional methods based on Mendelian inheritance worldwide. When a GM crop is cultivated in the proximity of a non-GM crop field, there will be gene exchange between them, whether it is low or high. This exchange is most possible between species belonging to Brassicaceae family, such as radish and OSR, with their high outcrossing rate (Brown & Brown 1996; see also Table 3). Vigouroux *et al.* (1999) reported that hybridization between bolting GM sugarbeet and weed beet occurred under field conditions. Colbach *et al.* (1999) simulated gene escape from GM OSR to volunteer OSR in time and space. Despite the predominance of non-GM OSR in the immediate locality, all sites was pollinated by a mixture of GM and non-GM sources (Bing *et al.* 1996; Thompson *et al.* 1999), suggesting that the farm-to-farm spread of GM crops will be widespread. Early isolation studies in western Canada indicated an average outcrossing rate using a chlorophyll-deficient marker from large fields to small plots of 0.6 and 3.7% at 366 m for *B. napus* and *B. rapa* respectively (Downey 1999). Although field-to-field crossing levels were low, a considerable number of hybrid seeds could remain in a field after harvest. Downey (1999) exemplified that a gene flow of 0.4% into a field yielding the Canadian average of 1400 kg/ha, with a harvest loss of 5% could result in some 70 000 outcrossed seed per ha remaining (seven seeds/m²) in the recipient field. If the hybrid is resistant to a herbicide and careful rotation and herbicide management practices are not followed, field-to-field gene flow can result in gene stacking. Thus, gene flow from GM to non-GM crops may result in genetic

contamination and also make its wild species weedier due to transgenes expressing resistance to herbicides or abiotic/biotic stresses.

Risks of gene flow between species

Of greater importance is the possibility of herbicide-resistant genes escaping to closely related weedy species. There is a risk that crops that are sexually compatible with wild relatives growing in proximity to them will receive fitness-enhancing transgenes such as herbicide- or insect-resistant genes, which could alter ecological parameters. Some examples are rice (Langevin *et al.* 1990), sorghum (Paterson *et al.* 1995), sugarbeet (Bartsch & Pohl-Orf 1996) and sunflower (Whitton *et al.* 1997). Other crops that have compatible relative species are also shown in Table 3.

In the case of crops with a high outcrossing rate, the largest concern in the USA and Europe has been over GM OSR, a crop with numerous wild relatives and increasing worldwide cultivation (Table 1). Significant barriers to the introgression of *Brassica napus* marker genes, as well as herbicide-resistant genes, into the genomes of *Raphanus raphanistrum* and *Hirscheldia incana* have been found (Anderson 1997; Chevre *et al.* 1997; Darmency *et al.* 1998). However, one hybrid was obtained from each of the crosses, *B. napus* × *Erucastrum gallicum* and *B. rapa* × *E. gallicum* (Downey 1999), indicating that the possibility of gene transfer from *B. napus* to *E. gallicum* exists, although the probability of its occurring is very low. In the case of inbreeding crops, rice and wheat are the most widely cultivated worldwide. It is reported that the 22 species of wild rice are distributed throughout Asia, Australia, Oceania and Latin America (Vaughan 1994; Bellon *et al.* 1998). Two wild species, *Oryza rufipogon* and *Oryza nivara*, are abundant in many parts of Asia and are known to hybridize with *Oryza sativa* under natural conditions (Cohen *et al.* 1999; Table 3). Numerous types of weedy rice also occur in Asia, derived from *O. sativa*, wild species, and hybrids between wild rices and *O. sativa* (Oka 1991; Suh *et al.* 1997). Considering (i) the occurrence and environmental persistence of hybrids between cultivated, weedy and wild rices; (ii) the extensive area of land that may eventually be planted with GM rice; and (iii) the large populations of wild and weedy rices in many rice-growing areas, it must be assumed that transgenes will escape to wild and weedy relatives (Cohen *et al.* 1999). Recently, two imazamox-resistant hybrids from a cross between *Aegilops cylindrica* and imazamox-resistant wheat were discovered in a research plot (Seefeldt *et al.* 1999; Zemetra & Jones 1999). The pro-

duction of this hybrid and the low level of self-fertility indicate that hybrids could serve as a bridge in the gene transfer between wheat and *A. cylindrica* in the field (Mallory-Smith *et al.* 1999).

Risks of gene flow between higher plants and other organisms

There is a strong likelihood that transgenes conferring pest resistance will be transferred from agronomic ecosystems into natural ecosystems, resulting in ecological risks creating more invasive weeds and affecting beneficial insects. Gene flow of the transgene to an infecting virus by recombination could lead to new viral genomes (Teycheney & Tepfer 1999). Analysis of viral genomic sequence data often shows genetic features that can be best attributed to RNA recombination events between viruses (Revers *et al.* 1996) or between viruses and plant RNA (Mayo & Jolly 1991; Masuta *et al.* 1992), indicating that RNA recombination is a normal feature of virus evolution. Thus, the use of viral sequences in transgenic plants immediately raised the questions of whether recombination could occur between viral transgene sequences and the genome of an infecting virus, and whether this could have an impact on virus evolution (Teycheney & Tepfer 1999). Wintermantel and Schoelz (1996) showed that the inoculation of transgenic *Nicotiana bigelovii* plants generated recombinant viruses that had a distinct competitive advantage in *N. bigelovii* when compared with the parental strain, demonstrating that gene flow from GM crops to infecting viruses can occur, although at very low levels and under particular conditions.

CONCLUSION

Continuous use of a single herbicide has been most responsible for herbicide resistance evolution in many cases (e.g. Powles *et al.* 1998). There have been more than 70 resistant weed species recorded since 1957 when the first incidence of herbicide resistance to 2,4-D was reported in *Commelina diffusa* and *Daucus carota*. Introduction of a GM crop resistant to a specific herbicide could result in accelerated evolution of herbicide resistance in weeds. Glyphosate use alone will clearly further engender the evolution of glyphosate resistance (Gressel 1996; Powles *et al.* 1998), and/or bring about a shift in weed spectra towards weeds that have never been controlled by glyphosate (Owen 1997). For resistance management, Gressel (2000) proposed that one way to delay the evolution of herbicide resistance in weeds is to stack two herbicide resistance genes in a GM crop and to use a mixture of herbicides with dif-

ferent modes of action. This may be useful because it considerably lowers the mutation frequency for resistance in the weed. However, a recent survey has shown widespread introgression of herbicide resistance traits among the various herbicide-resistant volunteer OSR populations in western Canada, resulting in many volunteer OSR populations already containing multiple resistance to all three herbicides on which herbicide-resistant GM OSRs rely (i.e. acetolactate synthase-inhibiting herbicides, glufosinate and glyphosate; Hall *et al.* 2000). A regulatory system could not prevent the re-introduction of triazine-resistant cultivars, and possibly could not prevent the use of 2,4-D-resistant cultivars if such were generated using known transgenes, because each case may be considered on its own merits (Gressel, 2000). This soon could become volunteer weed population of OSR in subsequent crops in rotational cycles. In addition, as shown in Tables 1 and 2, some efforts have already been made to introduce multiple traits in a single crop cultivar. If a GM crop cultivar with stacked genes for multiple resistance to herbicides and other pests is released into natural ecosystem and remains there, the GM crop cultivar itself could be a mighty weed that cannot be controlled by any herbicides available in the farmers' arsenal.

In favor of GM crops, GM technology can contribute to supporting increasing global populations, particularly of developing countries. This benefit seems to overwhelm potential risks when only considering present statistics, but we cannot conclude that GM crop is safe enough for us not to worry about its risks. Zero risk does not exist in many cases, but our concern is whether the risk is acceptable. So far, the long-term consequences have not well been quantitated. Gene flow is affected by the various factors mentioned above, so more comprehensive and systematic approaches will support the right decision for GM crops, and indeed whether any particular GM crop cultivar is acceptable. A recent study by Neve *et al.* (2000) gives us a hope that long-term gene flow from GM crop can be simulated. To establish better strategies for minimizing risks arising from GM crops, more studies on weed biology and ecology are essential.

In conclusion, we hope that this discussion will induce researchers to assemble available data, conduct more basic studies to collect fundamental information on the biology and ecology of crops and weeds in agro-ecosystems, and make the information accessible to others for better risk assessment and management.

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