

Potential for the environmental impact of transgenic crops

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In recent years, there has been increasing interest in how changes in agricultural practice associated with the introduction of particular genetically modified (GM) crops might indirectly impact the environment. There is also interest in any effects that might be associated with recombinant and novel combinations of DNA passing into the environment, and the possibility that they may be taken up by microorganisms or other live biological material. From the current state of knowledge, the impact of free DNA of transgenic origin is likely to be negligible compared with the large amount of total free DNA. We can find no compelling scientific arguments to demonstrate that GM crops are innately different from non-GM crops. The kinds of potential impacts of GM crops fall into classes familiar from the cultivation of non-GM crops (e.g., invasiveness, weediness, toxicity, or biodiversity). It is likely, however, that the novelty of some of the products of GM crop improvement will present new challenges and perhaps opportunities to manage particular crops in creative ways.

Debates about the commercial introduction of GM crops in some parts of the world have led to questions about their potential impact on the environment. Could they affect non-target insects, outcross to produce uncontrollable weeds, or have adverse effects on wildlife? To what extent could they benefit the environment by reducing certain chemical inputs into agriculture or providing raw materials currently obtained from non-renewable sources? Although it is acknowledged that agriculture inevitably has an impact on the environment, what is a reasonable trade-off between crop production, wildlife, and consumer concerns?

The assessment of the environmental impact of GM crops is a fundamental part of the international regulatory process undertaken before GM crops can be grown under field conditions experimentally or commercially. As a consequence, it has been the subject of research over the past 15 years¹. In 2001, 52.6 million hectares (130 million acres) of GM crops were grown commercially, and there has been extensive experience of GM crops in commercial agriculture in over ten countries².

The objective of this review is to outline what is known about the different kinds of environmental effects of GM crops and to discuss the principles and questions that have emerged from the assessment of environmental impact so far. To consider the present state of knowledge is timely, not least because Europe is in intense discussions about whether to commercialize GM crops, some of which have been in the regulatory pipeline for several years. The literature on the topic is extensive, so we will illustrate the nature of potential environmental impacts with examples. We also aim to identify gaps in knowledge and provide signposts to areas where further research is needed.

One of the most challenging issues in assessing environmental effects is in deciding what constitutes an acceptable or unacceptable environmental impact. Discussions of this type tend to step outside the realms of science into value judgments about the relative importance of different living things. A question often asked is: should the

environmental impacts of GM crops be compared with those from conventional, organic, or integrated farm management practices? This is a fascinating and important area of debate, but it is outside the scope of this review. For our purpose here, environmental impacts will be compared against a baseline of non-GM conventional agricultural practice that is the predominant form of agriculture internationally.

We concentrate on the scientific analysis of environmental impact and do not discuss socioeconomic or ethical issues. The primary questions about environmental impact are the same regardless of the method of plant breeding used: what is the nature of the genetic change, how is the genotype and phenotype of the crop modified, and what is the environmental consequence of that change? We have divided the topic to consider two issues: direct effects of the crop on the environment, and indirect effects on the environment by changing agricultural practice (Fig. 1). The fate and impact of naked DNA in the environment is also considered (see "Fate of free DNA in the environment").

Toxicity to living things

Non-target effects are defined as undesirable effects of a novel gene (usually conferring pest or disease resistance) on "friendly" organisms in the environment. Identifying a resistance gene and targeting its product to appropriate plant tissues so that it acts only against the pest, without adverse effects on friendly organisms, is challenging. This is particularly true where the benign or beneficial organism is related to the pest to be controlled and shares similarities in physiology. Targeting the pest without undesirable side effects is a potential problem for all methods of control, whether chemical or biological.

The use of toxins by plants for defense against pests and diseases is a common phenomenon in nature. Some natural defense substances can be highly toxic, such as glycoalkaloids in deadly nightshade (*Atropa belladonna*) and ricin, found in the castor bean (*Ricinus communis*). Transgenic pest-protection strategies generally depend on

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Table 1. Potential impacts* of GM crops on the environment

Class	Example
Direct impact of novel traits on the environment	
Chemical interaction with living things	Non-target effects of insect resistance Fate and consequence of insecticidal toxins in soil
Change in persistence or invasiveness of the crop	Persistence in agricultural habitat (weediness) Invasiveness in natural habitats
Gene flow by pollination to weeds and feral plants	Transfer of herbicide tolerance to weeds Transfer of biotic and abiotic stress tolerance to weeds or feral species Stacking of herbicide tolerance genes
Indirect impact of changing agricultural practice on the environment	
Reduced efficiency of pest, disease, and weed control	Development of weeds tolerant to herbicides by evolution and selection from within the weed gene pool Development of resistance to <i>Bt</i> toxins in pests
Effect on wildlife biodiversity	Effects of broad-spectrum herbicides
Effect on soil and water	Change in herbicide use Change in soil cultivation patterns

*Impacts can be detrimental or beneficial. Risk assessment focuses primarily on the analysis of potential negative effects on the environment.

the transfer and expression of novel or natural defensive plant genes³. The most extensively studied examples of engineered resistance are based on the use of delta endotoxins of the bacterium *Bacillus thuringiensis* (*Bt*). *Bt* is a naturally occurring ubiquitous soil bacterium that produces a toxin lethal to certain insects.

One of the most significant studies of non-target impacts has been on the effects of *Bt* insecticidal proteins on the monarch butterfly in the United States. A laboratory study published in 1999 suggested that "*Bt* maize" was a potential hazard to monarch butterfly larvae consuming milkweed leaves (*Asclepias* spp.) with pollen from corn containing a *Bt* gene sprinkled on the milkweed leaf surface⁴. This initial study did not assess the ecological consequences of these findings. It measured the toxicological response of monarch larvae to the toxin, but did not investigate the level of exposure of the insect to the toxin in natural field conditions. This report generated worldwide interest and, in consequence, a collaborative research program was established in 1999 (ref. 5). The research focused on acute and toxic effects of *Bt* corn pollen and the degree to which monarch larvae would be exposed to toxic amounts of *Bt* pollen by feeding on milkweed plants found in and around cornfields. The authors of this study reported that, although *Bt* pollen does have some toxic effects on butterfly larvae, the pollen densities likely to be encountered are low and pose negligible risk to the monarch.

It has been observed, however, that the toxic properties of the *Bt* maize pollen differ among *Bt* maize lines. The transformation events with the highest expression of *Bt* have the greatest adverse effect on the monarch⁶. It is likely that in the future, greater attention will be given to gene promoters of *Bt* and transformation events that lead to negligible or no *Bt* expression in pollen. An ideal pest resistance mechanism is sharply targeted so that it inhibits the pest of interest and has limited non-target effects on other herbivores and organisms in the environment. Assessments may need to be made of the possibility that the ecological niche vacated by a primary crop pest could be filled by a secondary herbivorous pest.

Other laboratory and field studies have looked at the effects of GM *Bt* crops on pest predators or parasites. In most cases, these studies measured the development and survivorship of insect predators reared on herbivore prey that had ingested corn leaves expressing *Bt*

toxins. Research by Hilbeck *et al.*^{7,8} reported the following: first, lacewings reared on *Ostrinia nubilalis* (European corn borer, ECB) or *Spodoptera littoralis* that had ingested corn leaves expressing *Bt* Cry1Ab toxin showed increased mortality and delayed development; and second, high concentrations of Cry1Ab in an artificial diet fed directly to lacewing larvae were toxic. These laboratory studies also emphasize the difficulties of conducting ecologically relevant laboratory experiments. The dose of toxin used in the final experiment was 30-fold higher than that found in most corn tissues in the field. Moreover, exposure in the field is not continuous as the predator has a choice of other insects or eggs to feed on. In the case of the ECB/lacewing study, the larvae in the field died soon after eating *Bt* corn plants. Any surviving larvae would normally feed inside the corn plant for most of their lives and would therefore not be available for consumption by lacewings⁹.

Caution must be used when drawing conclusions from results of small sets of

laboratory studies that test non-target species under unnatural conditions or focus on hazard alone, without considering the level of exposure that occurs under natural conditions. The type of effect on natural enemies of plants depends as much on the specific attributes of the resistance mechanism as it does on the details of the interactions among the target and non-target species—that is, their host/prey interaction, and the plants on which this interaction occurs¹⁰.

The issue of baseline environmental impact is particularly relevant here because there is good evidence that the pesticidal sprays used on maize may be more harmful to the monarch butterfly than is *Bt* corn pollen¹¹. In evaluating the use of *Bt* crops and their possible environmental damage, it is important to take into account the environmental damage caused by the use of pesticides in agriculture generally. It is argued that millions of birds and billions of insects, both harmful and beneficial (including pollinators and biological control agents), are killed each year in the United States alone as a result of pesticide use.

Widespread use of chemical pesticides to control primary pests often disrupts the natural controls that prevent the outbreak of secondary pests by destroying natural insect enemies. If the planting of GM pest-resistant crop varieties eliminates the need for broad-spectrum insecticidal control of primary pests, naturally occurring control agents are more likely to suppress secondary pest populations, maintaining a diversity and abundance of prey for birds, rodents, and amphibians.

It has also been proposed that the incorporation of resistance to potato leaf roll virus may preclude the need to spray against the aphid vector of the virus³. It is expected that as a consequence of allowing the number of aphids to increase, the number of natural control beetles (ladybirds) would also increase. This kind of application of disease resistance may have a direct benefit in increasing the numbers of natural pest predators in agro-ecosystems.

The fate and consequences of insecticidal toxins in soil is also an important consideration. It has been shown that *Bt* plants exude *Bt* toxins from their roots during their entire life cycle, and that the toxins are also released from dead plant material incorporated into the soil after harvest¹². *Bacillus thuringiensis* is a ubiquitous soil-borne

bacterium that naturally releases *Bt* toxins into soil. But *Bt* toxins from crop sources are likely to result in the additional exposure of soil organisms to these toxins. Saxena and Stotzky¹³ investigated the effect on soil organisms of Cry1Ab toxin released from roots and from crop residues. They found that the toxin was present in the guts and casts of tested earthworms. But there were no significant differences in mortality or weight of these organisms or in the total number of other soil organisms (including nematodes, protozoa, bacteria, and fungi) between the soil rhizosphere of *Bt* and non-*Bt* crops.

The *Bt* toxin can bind to elements within the soil (such as clay particles or humic acids), become stabilized¹⁴, and remain active for possibly hundreds of days⁹. As the toxin needs to be ingested to be active against insects, it is likely that toxins bound to soil particles would potentially threaten only those organisms that feed on soil, such as earthworms. The *Bt* Cry proteins have been tested against a wide variety of terrestrial and aquatic invertebrates, including earthworms, collembola, daphnids, insect predators and parasites, spiders, and honeybees. In most cases, no adverse effects were observed, even when test populations were exposed to toxin concentrations over 500–1,000-fold greater than those they would be expected to encounter under field conditions⁶.

The studies determining rates of degradation of Cry proteins in soil were mostly carried out in soil microcosms. But Head *et al.*¹⁵ have also investigated the fate of *Bt* toxins in soil under field conditions. Their study measured the level of Cry1Ac protein in soils collected from inside and outside fields where *Bt* cotton had been grown and the crop subsequently incorporated into soil by post-harvest tillage for three to six consecutive years. The amount of protein was evaluated using enzyme-linked immunosorbent assay and insect bioassays. Data showed that the amount of Cry1Ac protein in the soil was below the level of detection for both test methods and did not result in detectable biological activity.

Persistence or invasiveness of crops

It is also important to determine whether newly introduced traits make a crop more likely to be more persistent (weedy) in agricultural habitats or more invasive in natural habitats. The definition of weeds, invasive plants, and colonizers, together with their ecological characteristics, have been discussed by Williamson¹⁶. The characteristics that define plants of these kinds remain obscure. Luby and McNichol¹⁷ and Baker¹⁸ stated that weediness arises from many different characters and that the addition of one gene is unlikely to cause a crop to become a weed. Both Fitter *et al.*¹⁹ and Williamson *et al.*²⁰ oppose this statement and suggest that small genetic changes can cause large ecological alterations. Despite the debate, there is consensus that if a crop species has very few weedy characteristics, the addition of one or a few genes would be unlikely to cause the crop to become a weed problem. Special attention must be given to crops that already have weedy characteristics or in which added genes might be expected to improve crop competitiveness in agricultural or natural habitats²¹.

Many plant species can be found both as a crop and as a weed²². A change in habitat may potentially result in the evolution of a weed from a cultivated plant or from a feral plant that is closely related to a cultivated species¹⁹. Various authors^{3,21,23,24} have observed examples of this in and around cultivated land.

As a high proportion of current GM crops carry tolerance to a particular herbicide, these crops have been the most extensively studied. The current scientific evidence indicates that, in the absence of herbicide applications, GM herbicide-tolerant crops are no more likely to be invasive in agricultural fields or in natural habitats than their non-GM counterparts^{21,25–29}.

The GM crops produced to date show little evidence of enhanced persistence or invasiveness. But the kinds of GM crops used in agri-

culture so far, in biological terms, have been limited. Some of the biological changes possible, such as changes in tolerance to extremes of temperature, water, soil salinity regimes, the introduction of pest or pathogen resistance, and changes in seed dormancy and propagation characteristics, could potentially have significant effects on persistence and invasiveness. It is important, therefore, that we continue with a rigorous case-by-case assessment of GM crops, accompanied by relevant underpinning research.

Gene flow from crops to related species

GM crop plants may be able to hybridize with sexually compatible species and have an impact on the environment through the production of hybrids and their progeny. There are four basic elements in determining the likelihood and consequences of gene flow in this way: first, the distance of pollen movement from the GM crop; second, the synchrony of flowering between the crop and pollen recipient species; third, the sexual compatibility between crop and recipient species; and fourth, the ecology of the recipient species.

There is now extensive literature on the distance of pollen movement in different crops³⁰. Some projects are engaged in mathematical modeling of pollen movement, and the general conclusion from the work to date is that pollination declines sharply with distance from the pollen source, but the distance at which pollination is zero is very difficult to determine. The distance viable pollen can travel is influenced by the pollen dispersal mechanism (wind, insects, etc.) and by pollen longevity, both of which are determined by plant species. Pollination distances of many kilometers have been recorded in some species³¹.

The sexual compatibility of crops with weeds and feral species has been reviewed by Ellstrand *et al.*³². He gives various examples of crops that can hybridize with weeds in some countries, including oilseed rape, barley, wheat, and beans. In the United Kingdom, the probability of hybridization with weeds is considered minimal for wheat, low for oilseed rape and barley, and high for sugar beet³³.

The possibilities of hybridization between oilseed rape and related species have been reviewed by Scheffler *et al.*^{34,35}. Several detailed studies of hybridization between oilseed rape and particular weed species have also been undertaken. Oilseed rape can hybridize with hoary mustard, with wild radish³³, and with other wild brassicas species^{3,34,35}.

The consequences of the transfer of novel genes from GM crops to weeds depend on the nature of the novel gene and the biology and ecology of the recipient weed species. The transfer of herbicide tolerance is unlikely to confer any competitive advantage to hybrids outside agricultural areas. The transfer to weed species of characteristics such as resistance to particular pests and diseases or tolerance to stressful conditions (such as drought or salt tolerance) could potentially give weed species a selective advantage. In this context, Ramachandran *et al.*³⁶ have investigated the competitive ability of an insect-resistant transgenic oilseed rape variety as compared with non-transgenic oilseed rape in seed mixtures. The transgenic variety was competitively superior when the two varieties were subject to diamondback moth selection pressure in greenhouse experiments and in field plots. In a similar study, Stewart *et al.*³⁷ have shown, under certain conditions, the likelihood of increased fitness in oilseed rape varieties expressing *Bt* transgenes.

Some of the modifications made by GM plant breeding are likely to have direct parallels in conventional plant breeding. Therefore, experience of the impact of hybridization between conventional crop varieties and weed species in conventional agriculture may be highly instructive in regard to the interpretation of the impact of hybridization between GM crops and weed species. However, some possible future genetic modifications in GM plant breeding, such as salt tolerance or cold tolerance, could potentially produce novel crop

Fate of free DNA in the environment

A novel DNA sequence encoding a trait could, in theory at least, enter the wider environment by several means. For example, plant DNA is present in soil or compost as a result of decaying plant residues. If a crop were GM, then the soil residue could also contain DNA encoding the novel trait. Similarly, the application of manure from animals eating a diet containing fodder from a GM plant could provide a route for the transfer of novel genes. Possible wider environmental impacts could also include the release of novel DNA in animal-derived products, for example, meat and milk used in the preparation of animal feed from GM crops.

To gain perspective when assessing the potential environmental impact of DNA from GM crops, it is important to consider the amount of DNA in the environment from non-GM origins. Pollen, leaves, and fruit alone result in thousands of tons of DNA per year being released into the environment⁶², in addition to the contribution from decaying plant and animal matter and release from microbes. It is important to emphasize that many of the genes being incorporated into GM crops are also present naturally in the environment⁶³. The amount of DNA derived from GM crops as a proportion of the total is therefore likely to be small, even if such crops are grown on a commercial scale.

The longevity of DNA in soil depends on various factors, including soil type and the presence of soil nucleases that degrade DNA. Laboratory microcosm experiments show the degradation of all but 0.1% of the target DNA sequence from transgenic tobacco within 40 days⁶⁴. A separate study involving the addition of free plasmid DNA to a microcosm showed the degradation of the polymer to below the limit of detection within five days. In addition to questions about the relevance of microcosm studies to the field situation, such studies also do not demonstrate the transforming capacity of the DNA for competent bacteria, on which its possible environmental impact depends.

The persistence of DNA in soil in the field seems to vary. Trace amounts of DNA from a transgenic tobacco trial were detected for several months in the field⁶⁵, whereas less than 0.4% of the target sequence of a different trial remained after two weeks⁶³, and small amounts of DNA remained on a site of transgenic sugar beet after two years. None of these studies, however, detected incorporation of a foreign gene into indigenous soil bacteria.

Few studies have investigated the persistence of DNA in compost, although bacteria carrying plasmids have been detected⁶⁶ and gene transfer has been observed⁶⁷. Similarly, there is a paucity of data on the survival of DNA in manure, although intact plasmids surviving in pig manure have been reported^{68–70}, making DNA transfer following uptake at least theoretically possible.

The possible presence of genes in manure depends largely on the survival of DNA as it passes through the digestive tract of an animal

fed a diet containing GM material. Limited studies to date have found no evidence of plant-derived DNA in manure from chickens or cattle fed grain or silage from *Bt* maize⁷¹. This may be due to degradation of the gene by nucleases in the animal gut^{72,73}.

Transfer of genes via animal feed derived from GM crops must also be considered in terms of possible wider environmental impact. On the basis of evidence to date, survival through the entire gastrointestinal tract seems unlikely. For example, transgenic DNA from glyphosate tolerant soybeans incubated in simulated gastric fluid was completely degraded within 15 seconds⁷⁴.

Even if "free" DNA does persist in the gut long enough to be taken up by bacteria, the possibility of gene transfer with other gut microbes should be considered. We know of no *in vivo* studies of this in ruminants, but gene transfer has been observed between cultured rumen bacteria in the laboratory^{75–77}. It is, however, difficult to extrapolate these studies to what happens *in vivo*, but the nuclease activity in the rumen⁷⁸ will cause DNA degradation, making the survival of intact genes in the rumen unlikely.

There is no evidence suggesting that functional genes are incorporated into meat products from animals fed GM feed. This is worthy of consideration, as decaying carcasses, for example, could be a possible source of the transfer of novel genes into the environment. Although plant-derived, non-transgenic DNA was detected in the meat from chickens on a diet of whole, unprocessed *Bt* maize, the *Bt* transgene was not found in the meat. Eggs and milk are another possible route for gene transfer, but the evidence to date shows no transgenic material in either of these products resulting from animals fed on GM material⁶⁵.

We have also considered the possibility of gene transfer in the preparation of animal feed. Aside from the obvious issues of segregation at the point of harvest and processing, DNA survival during feed preparation is also an important issue. It seems that only feed that has undergone heat processing, chemical expulsion, or extrusion contains DNA degraded to the point that it can no longer act as a source of functional genes^{79,80}.

Silage is an important animal feed, and will also contain intact plant genes⁷⁹. Fragments of transgenic DNA persisted in silage made from *Bt* maize for up to 106 days while shorter, nonfunctional fragments survived for up to 7 months after ensilage⁸¹. The possibility of effluent from silage fermentation containing functional genes is low; plasmid DNA was undetectable after exposure to silage effluent for just 30 seconds. Further studies on the survival of non-GM maize chromosomal DNA in silage effluent showed degradation of the DNA after one minute⁸².

types whose impact on the environment will need to be assessed with particular care.

It is generally assumed that there is likely to be an environmental impact only if the novel trait acquired confers greater environmental fitness on the crop plant or its sexually compatible relative. This is not necessarily the case. A gene that confers reduced fitness on a plant in a wild habitat could adversely affect wild sexually compatible natural populations through recurrent pollination by a GM crop³⁸.

Another matter of importance is the potential of crops carrying different herbicide-tolerance genes to become multiply tolerant to several herbicides by pollination between adjacent crops. In several closely studied examples in Canada, farmers have detected oilseed rape plants tolerant to three different herbicides³⁹. Two of the toler-

ances were acquired from GM crops and the third from conventional breeding. Various studies are ongoing in Europe to define management practices that will minimize the likelihood of this occurring. Unless agronomics and weed control measures are managed well, the development of multiple tolerance in "volunteer" crop plants (from seeds remaining viable in agricultural soil) may also impact the environment by necessitating the use by farmers of less environmentally friendly (and possibly outdated) herbicides.

Reduced efficiency of pest, disease, and weed control

Changes in agronomic practice associated with the introduction of particular GM crops could also have potential impacts on the environment. These include the increased use of broad-spectrum herbicides, the more frequent sowing of a crop (e.g., winter sowing

instead of spring), or the increased use of minimal cultivation agriculture. Some of the indirect effects may be beneficial to the environment; for example, minimal cultivation generally reduces soil erosion and favors earthworms; here, however, we focus on potential negative effects on the environment.

Weeds resistant to herbicides can develop by evolution and selection from within weed populations continually sprayed with the same herbicide (rather than by gene flow from a herbicide tolerant crop). Weed species have historically displayed a remarkable ability to evolve tolerance to particular herbicides. Several studies have demonstrated that tolerance to a particular herbicide is often more likely to develop by evolution from within the weed gene pool rather than by gene flow from herbicide-tolerant crops^{40,41}. Glyphosate tolerance was considered to be highly unlikely to evolve in weed species in this way. However, there is now a well-characterized example in annual ryegrass in Australia⁴² and a recently published example in horseweed in the United States⁴³.

Farmers and plant breeders have for many years struggled to keep ahead of a pest's ability to adapt to the techniques used to control it. Experience with chemical pesticides has proved challenging, as pests evolve resistance to chemical pesticides^{44,45}. Pests have also historically overcome resistance introduced into crop plants by traditional breeding programs⁴⁶. More recently, several studies have shown that pests can also adapt to *Bt* toxins produced by biopesticide sprays containing *B. thuringiensis* under field and laboratory conditions⁴⁷. Pest resistance to GM *Bt* crops has been observed under laboratory conditions⁴⁸ but not, to our knowledge, under field conditions to date.

It is possible that the widespread use of *Bt* crops could lead to the evolution of several important insect pests that are resistant to the *Bt* biopesticide. This could potentially make it necessary to resort to less environmentally acceptable chemical pesticides. This is of particular concern to organic farmers because they use the *Bt* bacterium as a permitted pesticide. Several strategies for resistance management have been proposed to delay adaptation to *Bt* crops by pest populations⁴⁴. The most widely used is the "high-dose refuge" strategy, which has been implemented in North America⁴⁹. The implementation of effective resistance-management practices is crucial to obtain the greatest benefits from pest-resistant transgenic crops and to allow the continued effective use of *Bt* biopesticides. An important part of good resistance management is to sow a refuge area of the crop, an area of the total crop that is deliberately susceptible to the pest. Where resistance to *Bt* in insects is controlled by recessive genes, the presence of refuge plants reduces the probability of the evolution of insects with resistance to the *Bt* toxin.

The next generation of GM pest-resistant varieties is likely to contain additional insecticidal genes. This pyramiding strategy is expected to delay the evolution of resistance much more effectively than the presence of a single insecticidal toxin and may require smaller refuges⁵⁰. The cotton variety sGK containing two insecticidal genes, *cryIA* and *CpTI* (cowpea trypsin inhibitor), is being planted commercially in China. Other stacked *Cry1Ac* and *Cry2AB* *Bt* varieties are also being developed. Cross-resistance among the toxins is a potential risk to the use of pyramids, and Roush⁵⁰ suggests that 100% mortality of susceptible insects on the GM crop is more critical to delaying the onset of resistance.

It is important to emphasize that a recent survey conducted by US maize growers⁵¹ has shown that, in the year 2000, almost 30% of the farmers failed to comply with the refuge protocols designed to prevent or delay the emergence of insects resistant to *Bt* toxins. This rate of non-compliance could well increase the risk of plant resistance breakdown. There is to date no reported evidence of insect resistance to *Bt* crops under field conditions in the United States, although *Bt*-

resistant insects have been observed in areas where *Bt* biopesticides are sprayed on crops⁴⁹.

Effects on wildlife biodiversity

The widespread introduction of GM herbicide-tolerant crops may cause a shift in weed populations and thus reduce weed species diversity and ecosystem complexity in the GM field and on neighboring farms. Herbicides are widely used in conventional agriculture and few weeds are normally observed in many non-GM crops following standard herbicide treatments. It is argued, however, that because the currently available GM herbicide-tolerant crops confer tolerance to broad-spectrum herbicides such as glufosinate and glyphosate, their extensive use may reduce the diversity of weeds in agricultural habitats.

It is debatable how broadly applicable these herbicides will be in practice and it is unclear what their precise impact will be on weed populations in comparison with the range of herbicides (some relatively persistent) currently used in conventional agriculture. The Farm Scale Evaluations currently being carried out in the United Kingdom and due to report in 2003 are designed specifically to compare the impact on wildlife biodiversity of GM herbicide-tolerant crops with conventional crops of the same type⁵².

The precise impact of the introduction of herbicide-tolerant crops will depend on the rotational and agronomic practices adopted to manage them. The adoption of different herbicide-use programs may have different effects on plant and animal biodiversity in fields and field margins⁵³⁻⁵⁵. Radosevich *et al.*⁵⁶ predicted that shifts in weed species composition and abundance would be exacerbated with the consecutive use of the same herbicide, providing favorable conditions for growth of particular weeds, insects, and diseases. Weeds exhibit considerable plasticity and thus are able to adapt to a wide range of cultivation practices. Experience with conventional agriculture has shown that weed species composition will vary within the same crop, among different fields, and at different times of year⁵². Thus, weed population shifts are natural ecological phenomena in crop management and should not be viewed as exclusive to GM crops. The effect of changes in herbicide programs on plant and animal biodiversity in field and field margins are being investigated in the United Kingdom²⁷.

Effects on soil and water

It is sometimes argued that the use of herbicides will increase with the widespread introduction of GM herbicide-tolerant crops and that this will contribute to the contamination of soil and groundwater and have undesirable effects on plant and animal diversity. However, no significant change in the overall amount of herbicide use has been observed with the adoption of herbicide-tolerant crops in the United States⁵⁷. A small but significant decrease in herbicide use has been reported in the United States since the introduction of GM soybeans^{58,59}. An analysis by US soybean growers reported by Trewavas and Leaver⁶⁰ shows that 7.2 million pounds of other herbicides have been replaced by 5.4 million pounds of glyphosate. This substitution resulted in the replacement of herbicides that are at least three times more toxic and that persist nearly twice as long as glyphosate⁵⁷. Furthermore, the Canola Council of Canada⁶¹ reported the results of a study that shows that herbicide-tolerant oilseed rape eliminated the use of >6000 tones of herbicide in the 2000 growing season.

Another important benefit of the use of herbicide-tolerant GM crops in the US is that they facilitate zero-till agronomic systems, which contributes to a reduction in soil erosion. There are, however, still active discussions on the significance of these kinds of data and on the long-term impact of the use of broad-spectrum herbicides⁵².

Future perspectives

In considering the impact of agriculture on the environment, the variables are numerous. However, there is extensive experience to draw upon in past studies of the environmental impact of conventional agricultural crops. It is important to examine the different potential impacts of GM crops within the context of this wider knowledge. As a consequence of the discussions about the commercialization of GM crops in some parts of the world, questions are being asked that were rarely considered for conventionally bred crops. This is true even though the products of many methods of conventional plant breeding (e.g., induced mutation and hybridization with wild relatives) carry a high level of unpredictability. The questions currently being asked of GM crops frequently demand a degree of forensic precision that would be impossible to apply to the products of more traditional breeding.

To minimize potential adverse effects of pest and disease resistance in GM plants on benign and beneficial organisms, it is important to target an appropriate level of gene expression to the parts of the plant where the resistance mechanism is needed. Targeting pest or disease control to the primary pest or disease is a demanding prospect, whether using chemical or biological approaches. To improve targeting of gene expression, the range of tissue-specific gene promoters needs to be diversified. It is also important that assessments of the impact of resistance mechanisms are collected from ecologically relevant *in vivo* agricultural environments. Laboratory experiments are important in establishing the principles that might influence non-target effects on friendly organisms, but care needs to be exercised in extrapolating their significance to the wider environment. Field experiments for this purpose are not trivial and often require several years of data in different locations to gather meaningful data.

It is rarely possible to make generic judgments about the persistence or invasiveness in the environment of GM crops or their progeny. The novel genes inserted into GM crops to date have been restricted in their likely biological impact. In the regulatory process, all classes of GM crop, especially novel genetic changes, must be assessed on a case-by-case basis in the context of the geographical locations where they are to be grown. The plant characteristics that need particular attention are those that significantly improve tolerance to biotic and abiotic stresses. Changes in breeding system and seed characteristics also have the potential significantly to alter survival and invasiveness. Most studies to date on gene flow have concentrated on the likelihood of a novel gene being transferred to a related wild or weedy plant species. However, further studies are needed on the ecology of potential gene recipient populations to assess the consequences of the transfer of a particular transgenic plant character, such as resistance to disease or pests, or an increase in cold tolerance.

Management of resistance mechanisms for pests and diseases is often difficult, regardless of the mechanism of control used. The spread of corn blight (*Helminthosporium maydis*) across the United States in 1970 is a dramatic example of what can happen if the genetic basis of resistance is allowed to become too narrow. The experience with *Bt* crops has demonstrated the importance of the management of resistance in *Bt* maize crops. One of the significant features of GM technology is that it can make possible the use of similar pest- or disease-resistance mechanisms in a diverse range of crops. This could potentially lead to intense selection pressures on pests and diseases to overcome resistance.

The diversity of resistance mechanisms used commercially in GM crops has, to date, been limited; it would benefit from using a wider range of different approaches operating in the same crop (transgene pyramiding) and in different crops. The same principle of resistance management also applies to herbicide-tolerant crops. In this case, the use of different herbicide-tolerance genes in different varieties of the same crop species (e.g., oilseed rape) can lead by gene flow to volunteer

crop plants and weeds becoming tolerant to several herbicides. This could make it necessary to resort to the use of herbicides that are environmentally less acceptable. In the future, it will likely be even more important to identify ways of encouraging or enforcing good management of pest, disease, and weed control strategies in agriculture.

Another issue related to gene flow is whether novel DNA sequences in GM plants (in themselves) impact the environment in ways different from the tons of DNA passing into the environment from other sources. While many of the DNA sequences introduced into GM plants are already present in nature, the combinations and quantities of particular DNA sequences may change under exploitation of GM crops. However, the proportion of the DNA with novel combinations of sequences will always be a minute proportion of the whole (e.g., one novel gene construct introduced into a wheat plant with about 80,000 resident genes). To ascertain the significance of the presence of novel DNA combinations in the wider environment, it would be important to establish the approximate background levels of particular genes at present. There have been few studies directly comparing survival of identical genes encoded in organisms of GM and non-GM origins. Conclusions are therefore derived by extrapolation from field and microcosm studies that may not necessarily have been conducted identically. A systematic analysis of these issues would therefore be helpful.

In considering the fate and possible impact of novel DNA sequences in the environment through manure, compost, animal feed, and crop remains deposited in soils, the critical issue is whether the DNA survives long enough to be transferred to other organisms. If this happens in nature, the most likely route is through competent bacteria. However, our knowledge of the incidence of bacterial competence under field conditions is limited. Furthermore, techniques used to detect DNA give little indication of the functional capabilities of the DNA to act as a source of transforming DNA. If a DNA fragment does not retain functional integrity, it is unlikely to have any environmental impact.

We mentioned at the outset the question of how to determine the significance of an environmental impact. It can be difficult to obtain objective data on environmental impact that is a true reflection of what happens in an agricultural habitat. Even more difficult is the decision of whether a particular impact is acceptable or unacceptable. The problem is illustrated by the question: if a particular GM crop is found to reduce earthworm numbers (e.g., by 50%) and this is considered an unacceptable environmental impact, would it follow that conventional agricultural practices with a similar impact on earthworms should be restricted?

Discussions about baselines for comparison of GM crops have in some countries grown out of concerns that some conventional agricultural practices have had a significant negative impact on the environment. In the United Kingdom, for example, where over 70% of the land area is farmed in some way, there is clear evidence of a significant reduction of certain species of farmland birds over the past 30 years. The case-by-case analysis of the environmental impact of GM crops as part of regulatory assessment has considerable merit, but it can lack vision about indirect effects on the environment that may be associated with the introduction of particular crops.

There is the possibility that if GM crops provide a new dimension of control over pests, diseases, and weeds in a poorly targeted way, they will drive agriculture farther toward monoculture and the excessive control of the agricultural environment. In this scenario, control would be exploited primarily to maximize crop productivity at the expense of supporting a diversity of wildlife food chains (such as those of insects, seeds, weeds, or birds) and habitats in the environment. These impacts on the environment as we have discussed are not restricted to GM crops, but one of the biggest challenges for the future is to manage the introduction and widespread commer-

cialization of GM crops in a way that favors the environment. It will be necessary to provide incentives that will require GM crops to be combined with agronomic practices that nurture crop diversity, sound crop rotation, soil fertility, and wildlife biodiversity, and that minimize the impact of agriculture on the environment.

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Research Errata

Excision of selectable marker genes from transgenic plants

Peter D. Hare & Nam-Hai Chua

Nat. Biotechnol. **20**, 575–580 (2002)

In the June 2002 issue, the references in the footnote of Table 2 in the above review were printed incorrectly. The footnote should read: “^PShown to be functional in plastids^{23,51}.” We apologize for any confusion this may have caused.

Potential for the environmental impact of transgenic crops

Philip J. Dale, Belinda Clarke & Eliana M.G. Fontes

Nat. Biotechnol. **20**, 567–574 (2002)

In the June 2002 issue, the received date for the above review was printed incorrectly. The correct received date is 3 April 2002, not May 2002. We apologize for the error.

Molecular strategies for gene containment in transgenic crops

Henry Daniell

Nat. Biotechnol. **20**, 581–586 (2002)

In the June 2002 issue, the above review stated on p. 583 that “One way of accomplishing this (gene containment) is with a system dubbed “terminator technology,” which was patented several years ago by the United States Department of Agriculture (USDA; Germantown, MD), Cornell University (New York, NY) Purdue University (West Lafayette, IN and Iowa State university (Ames, IA), and licensed to Delta and Pine Land Company (D&PL; Scott, MS), which was subsequently bought by Monsanto (St. Louis, MO).” In fact, the three patents on which the Technology Protection System is based, Control of Plant Gene Expression (U.S. patents 5,723,765, 5,925,808, and 5,977,441), are jointly owned by D&PL and the USDA. This is because employees of D&PL and the USDA were the inventors of this technology. Second, Cornell University, Purdue University, and Iowa State University were never involved in the development of this technology and hold no ownership in it. Third, D&PL did not license the technology as it is a co-owner; instead, in July 2001, it signed with the USDA a licensing agreement that gave D&PL the exclusive right, and the responsibility, to market the technology to third parties and pay royalties to the USDA. Fourth, D&PL was not bought by Monsanto and remains an independent, publicly traded company (DLP on the NYSE). We apologize for the error.