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REVIEWS AND SYNTHESES

# Assessing environmental risks of transgenic plants

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## Abstract

By the end of the 1980s, a broad consensus had developed that there were potential environmental risks of transgenic plants requiring assessment and that this assessment must be done on a case-by-case basis, taking into account the transgene, recipient organism, intended environment of release, and the frequency and scale of the intended introduction. Since 1990, there have been gradual but substantial changes in the environmental risk assessment process. In this review, we focus on changes in the assessment of risks associated with non-target species and biodiversity, gene flow, and the evolution of resistance. Non-target risk assessment now focuses on risks of transgenic plants to the intended local environment of release. Measurements of gene flow indicate that it occurs at higher rates than believed in the early 1990s, mathematical theory is beginning to clarify expectations of risks associated with gene flow, and management methods are being developed to reduce gene flow and possibly mitigate its effects. Insect pest resistance risks are now managed using a high-dose/refuge or a refuge-only strategy, and the present research focuses on monitoring for resistance and encouraging compliance to requirements. We synthesize previous models for tiering risk assessment and propose a general model for tiering. Future transgenic crops are likely to pose greater challenges for risk assessment, and meeting these challenges will be crucial in developing a scientifically coherent risk assessment framework. Scientific understanding of the factors affecting environmental risk is still nascent, and environmental scientists need to help improve environmental risk assessment.

## Keywords

Environmental risk assessment, gene flow, genetic engineering, non-target effects, resistance management, tiered risk assessment, transgenic plants.

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## INTRODUCTION

The creation of the first transgenic organisms during the early 1970s initiated the debate about their risks that continues today. Stimulated by the 1975 Asilomar conference, molecular biologists made recommendations for the safe use of transgenic organisms in the laboratory (Berg *et al.* 1975) that led to the US National Institute of Health (NIH) guidelines for laboratory research on recombinant DNA (NIH 1976) and many discussions about the risks of transgenic organisms worldwide.

There was widespread recognition that some transgenic organisms would be used in the environment; however, environmental risk issues were not addressed effectively until the 1980s, when the debate was broadened to include ecologists, evolutionary biologists, epidemiologists, and others. At that time, microbial transformation had become routine, and plant transformation was considered a technology of the distant future. In 1982, the US Environmental Protection Agency (EPA) commissioned a report (Gillett *et al.* 1986) and the Oak Ridge National Laboratory developed a paper (Sharples 1982) on the environmental risks of transgenic organisms. In 1984, a meeting of prominent ecologists and evolutionary biologists was held at the Cold Springs Harbor Laboratories to discuss environmental risks (Brown *et al.* 1984).

This early debate centred on two issues. Were there potential environmental risks of transgenic organisms that merited assessment, and if so, how general or case-specific should the assessment be? Sharples (1982) and Gillett *et al.*

(1986) looked for possible risks of transgenic organisms, and concluded that for every type of transgenic organism considered, there were potential environmental risks. Although the emphasis at that time was on transgenic microorganisms, risks associated with plants were also of considered importance. Regal (1986) approached the issue theoretically. In an informative paper, he demonstrated that all arguments that purported to show that there were no substantive environmental risks of transgenic organisms were logically flawed. For example, to the claim that nature has already tried all possible genetic variants so genetic engineering is unlikely to create anything new, he noted that one human being alone can produce $10^{2017}$ kinds of gametes, which dwarfs the number of atoms estimated in the universe ($10^{70}$). Clearly, nature has not tried all possible genetic variants. In total, he showed that eight generic safety arguments and one generic risk argument were logically flawed (Regal 1986). By the mid-1980s, it was clear that there were substantive potential environmental risks of transgenic plants that required assessment. This became an important element in the USA in the Coordinated Framework for the regulation of biotechnology [Office of Science & Technology Policy (OSTP) 1986].

The results in these early works also implied that risk assessment should be conducted on a case-by-case basis (see references in Andow *et al.* 1987). This point was extended by a scientific committee of the US National Academy of Sciences [National Research Council (NRC) 1987], which concluded that case-specific risk assessments needed to consider the source and target environments, the biological and ecological characteristics of the transgenic organism, and the scale and frequency of introductions. The main alternative was to extend the NIH Guidelines to cover environmental release. The hope of the proponents of this idea was that transgenic organisms could be readily classified into a small number of risk categories. Each category would have associated a fixed, pre-specified set of risk management practices that would reduce the risks to acceptable levels. This alternative never gained scientific credibility among ecologists and other environmental scientists because classification of transgenic organisms into a small number of risk categories was unrealistic. Instead, the case-specific approach was further clarified in a statement endorsed by the Ecological Society of America (Tiedje *et al.* 1989), which summarized the findings of the 1980s and would contribute to the scientific basis for an international consensus that environmental risk assessment of transgenic plants was necessary and should be done on a case-by-case basis, taking into account some integrated understanding of the transgene, the recipient organism and the intended environment of release.

In this review, we concentrate on major scientific developments that have occurred after *c.* 1990 and have influenced the conduct of environmental risk assessment of transgenic plants. We do not draw conclusions about the level of risk of any transgenic plant, but concentrate on how environmental risk assessments have been and need to be improved to meet the present and future challenges of transgenic plants. In other words, we will not answer the question, 'How great is the risk of Bt maize (transgenic maize with genes from *Bacillus thuringiensis*) to the environment?' but will examine how research on the possible risks of, for example, Bt maize has affected the risk assessment process.

Classically, risk assessment follows four steps (NRC 1983; EPA 1998): hazard identification, exposure assessment, effects assessment and risk characterization. *Risk* is the probability that some adverse effect occurs from an environmental hazard (in this case, a transgenic plant with a transgene product), and is classically comprised of (1) the probability that the environment is exposed to the hazard (exposure assessment); and (2) the conditional probability that the adverse effect will occur, given such exposure (effects assessment). Hazard identification involves identifying the possible causes of the potential adverse effects, but is often expanded to include identification of the possible adverse consequences that could result from the identified hazards. Risk characterization synthesizes the information to estimate risk. These four steps provide a rough, but convenient framework for understanding the relation of scientific developments during the 1990s to environmental risk. In general, most of these main results relate to the identification of possible adverse effects.

The period from *c.* 1990 has seen the emergence of a broad scientific consensus around the kinds of environmental risks of transgenic plants. Although early reports indicated that transgenic plants had no new 'kinds' of environmental risks (NRC 1987; Tiedje *et al.* 1989), the 'kinds' remained unspecified. The kinds of environmental risks were first summarized by Snow & Morán-Palma (1997) as: (1) non-target and biodiversity risks, which include non-target species, ecosystem functions, and effects on soils; (2) risks associated with gene flow and recombination; and (3) risks associated with the evolution of resistance in the target organisms, such as insect pests to transgenic Bt crops and weeds to the herbicides applied to transgenic herbicide-tolerant crops. The third kind of risk is important presently because about 99% of all transgenic crops worldwide are Bt or herbicide-tolerant crops. Numerous subsequent reviews have supported these three kinds of risk (e.g. Wolfenbarger & Phifer 2000; NRC 2002; Snow *et al.* 2005). These 'kinds' of risk are not new, because many methodological approaches for assessing them are already known. This does not mean that all of the risks for any transgenic plant will be ones that have been assessed in the past, only that all

such risks can be assessed (NRC 2002). We will address scientific results that are influencing how risk assessment is conducted for each of these three kinds of risk in turn.

The new results have created instability in the international regulatory environment, which in turn has resulted in calls for agreement on a common conceptual framework for environmental risk assessment of transgenic organisms. Yet agreement remains unattainable (Strandberg *et al.* 1998). Despite this general lack of agreement, there is a broad consensus that tiering environmental risk assessment is essential to allocate effort to more serious risks while reducing effort on less serious ones. Considerable differences exist in how tiering should be structured, and we evaluate and synthesize the various suggestions and propose a practical framework for tiering environmental risk assessment for transgenic organisms.

Some of the major scientific issues that remain for the future include: exactly what integrated understanding of the transgene, recipient organism and environment is essential for environmental risk assessment; what is the appropriate comparison for evaluating risk; and what constitutes an environmental harm? We do not attempt to resolve these issues in this review, but provide a context from which they can be addressed in the future. In the final section, we address two issues with which environmental risk assessment of transgenic crops will need to grapple in the future, assessment of risks to biodiversity and assessment of the diverse transgenic crops anticipated in the future.

## NON-TARGET AND BIODIVERSITY RISKS

Non-target organisms are species that are not the target of a transgenic plant. All transgenic plants have some non-target species. These species can be grouped into several overlapping categories (Andow & Hilbeck 2004; Snow *et al.* 2005): (a) beneficial species, including natural enemies of pests (e.g. ladybird beetles, parasitic wasps) and pollinators (e.g. bees, bats); (b) non-target herbivores; (c) soil organisms; (d) species of conservation concern, including endangered species and charismatic species (e.g. monarch butterfly); and (e) species that contribute to local biodiversity. Biodiversity risks include any adverse effects on non-target species or ecological processes that affect biodiversity, which remains poorly defined.

Through 1997, most studies on non-target and biodiversity risks of transgenic plants showed no effects of the transgenic plant on non-target organisms (Fitt *et al.* 1994; Sims 1995; Dogan *et al.* 1996; Orr & Landis 1997; Pilcher *et al.* 1997; Yu *et al.* 1997; EPA 2001; Monsanto Company 2002a,b). Only one laboratory study showed lower survival of a non-target species, that being the springtail *Folsomia candida* (Willem) (Collembola, Isotomidae) when fed with high concentrations of Bt corn leaf protein (EPA 2001), although the connection to environmental risk remains unclear. Some of the species used in these early trials are not closely associated with the transgenic plant tested or the area where the plants are grown and are of questionable ecological relevance. However, based on these studies, many scientists believed that non-target species were not significantly at risk to transgenic crops.

In 1998, studies by Hilbeck *et al.* (1998a,b invigorated consideration of non-target hazards by reporting an unexpected adverse effect of Cry1Ab, the active product of Bt corn, on the predatory green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). They fed *C. carnea* larvae with prey that had consumed Bt corn or a diet containing purified Cry1Ab toxin, and found higher immature mortality compared with controls. These results were surprising because Cry1Ab is believed to be toxic only to Lepidoptera, while *C. carnea* belongs to the Neuroptera, an order that is more closely related to the Coleoptera than to the mecopteroid orders, which include the Lepidoptera. These results have been confirmed by additional studies (Hilbeck *et al.* 1999; Dutton *et al.* 2002a, 2003a; De Maagd 2004); although the mechanism is not direct toxicity (Hilbeck *et al.* 1999; Romeis *et al.* 2004), it is still uncertain (De Maagd 2004). These studies suggested that Cry toxins may be less specific than previously believed, and although this inference is still debated among scientists, it has contributed to the broadening of non-target risk assessment.

In the years following 1998, publications on potential non-target risks focused on species that might be at risk or cause environmental risks in the local areas where the transgenic plants were meant to be cultivated. This shift from assessing indicators of non-target risk to assessing actual risks is one of the major changes to occur in the late 1990s and early 2000s. This was brought fully into focus during 1999, when Losey *et al.* (1999) suggested that monarch larvae (*Danaus plexippus* L., Lepidoptera, Dandaidae) suffered higher mortality when feeding on their primary host plant, the common milkweed *Asclepias syriaca* L., dusted with transgenic Cry1Ab Bt pollen. This initial observation was confirmed by Jesse & Obrycki (2000) and coupled with the realization that *c.* 50% of the monarch breeding habitat is located in the US Corn Belt (Wassenaar & Hobson 1998), it triggered concerns that large-scale cultivation of Bt corn would harm the monarch population. Monarch butterflies are not endangered, but attract wide interest in the USA for many reasons, such as their beauty, iconic significance to the public, and spectacular migration over several thousand kilometres. Stimulated by these results, a group of researchers conducted a series of studies to estimate the risk of Bt corn in the USA to monarch butterflies (Hellmich *et al.* 2001; Oberhauser *et al.* 2001; Pleasants *et al.* 2001; Sears *et al.* 2001; Stanley-Horn *et al.* 2001). In one of the most thorough published non-target risk assessments of a

transgenic crop, Sears *et al.* (2001) concluded that the risk to monarch populations was insignificant, and in an excellent review, Oberhauser & Rivers (2003) summarized the events and findings associated with these studies. Recent studies (Anderson *et al.* 2004; Dively *et al.* 2004; Jesse & Obrycki 2004), however, have revealed a much higher toxicity of Bt pollen and anthers than found in previous studies. Although Dively *et al.* (2004) suggested that the risk to monarchs remains insignificant, a close analysis of the issues may allow alternative assessments related to different transformation events. In addition, uncertainties in the risk assessment have not been examined. Uncertainty is inherent in the concept of risk (Hill & Sendashonga 2003) and includes measurement uncertainties, uncertainties related to the conditions of observations, and inadequacies of models (NRC 1993).

The shift to considering actual potential risks in local environments, rather than indicators of risk, may have also led to assessment of risk to the Federally endangered Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov, Lepidoptera, Lyceanidae) in the USA, and subsequently to other endangered Lepidopteran species. Instead of focusing only on commercial corn fields, dispersal of pollen and the production of corn in wildlife refuges could expose this species to Bt pollen. The 2000 Scientific Advisory Panel of the EPA (EPA-SAP 2001) acknowledged the possibility that this species may come in contact with Bt pollen, and requested additional assessment of the risks to Karner Blue butterfly. Similarly, studies on the possible exposure and higher mortality of two protected butterfly species in Hungary, *Inachis io* L. and *Vanessa atalanta* L. (Lepidoptera, Nymphalidae; Darvas *et al.* 2004), and the lack of effects on black swallowtail *Papilio polyxenes* F. (Lepidoptera, Papilionidae) under field conditions (Zangerl *et al.* 2001), have been motivated by consideration of the local risk directly to those butterflies rather than indicators of risk.

After 1999, potential effects and exposure routes associated with soils were identified. Saxena *et al.* (1999) found that Cry1Ab is released into the soil via corn root exudates, where it can persist for at least 350 days (Saxena *et al.* 2002). These results suggested that Bt corn could possibly affect rhizosphere and soil communities. Later, Zwahlen *et al.* (2003a) reported that the Cry1Ab toxin in Bt corn litter persisted for at least 8 months. Together these studies showed that long-term exposure of soil organisms to Bt toxins was possible and that the risks of Bt crops on the soil biodiversity and soil ecosystem functioning should be assessed. However, Zwahlen *et al.* (2003b) showed that mortality and weight development of adult and juvenile earthworms, *Lumbricus terrestris* L. (Oligochaeta, Lumbricidae), were not significantly different when fed Bt or non-Bt corn residues, with the exception that after 200 days, adults fed Bt corn residues had a significant weight loss compared with those fed non-Bt corn. For earthworms, if Bt corn has an adverse effect in local agricultural environments, it will likely be difficult to detect with small scale experiments. Although Head *et al.* (2002) did not find any persistence of Cry1Ac or Bt cotton plant residues in the soil 3 months after full tillage, cotton conservation tillage systems, which are common and have higher residue persistence, have not been studied.

Concerns about a loss in biodiversity associated with the cultivation of genetically modified crops had been expressed by Krebs *et al.* (1999) who argued that any potential adverse effect of transgenic crops is layered onto a biodiversity landscape that is already severely damaged by the intensification of agriculture. However, such large-scale risks were hardly studied until 2000, when Watkinson *et al.* (2000) suggested that the cultivation of herbicide tolerant crops might adversely effect skylark populations in the UK. A large-scale field evaluation of herbicide-tolerant crops in the UK was established to quantify actual effects on non-target species (Brooks *et al.* 2003; Champion *et al.* 2003; Firbank 2003; Haughton *et al.* 2003; Hawes *et al.* 2003; Heard *et al.* 2003a,b; Roy *et al.* 2003; Squire *et al.* 2003; see also Perry *et al.* 2004; Strandberg *et al.* 2005). For the most part, ecological effects of herbicide-tolerant crops probably propagated from whatever changes in the weed community that occurred from a change in herbicide use. A summary of the major findings of these studies is given by Andow (2003) and Freckleton *et al.* (2003). The risk implications of these results have been difficult to draw because they depend on the farmers' responses to current economic conditions (Watkinson *et al.* 2000; Squire *et al.* 2003).

Lövei & Arpaia (2005) reviewed the results of 44 laboratory experiments evaluating the effect of transgenic crops on arthropod natural enemies (18 species of predators and 14 species of parasitoids). All but one of these studies was published during or after 1998. Even though many of the studies had small sample size and/or large error variance, a remarkable 35.0% of all response parameters measured were significantly negatively affected by the transgenic crop. We conclude that there is a significant need to develop and improve existing risk assessment methodologies to enable clear and rapid assessment of potential risks to non-target species.

To systematically address the shifts since 1998, Andow & Hilbeck (2004) outlined a risk assessment model for non-target species that could occur in particular local agricultural environments from a specific transgenic plant. Which non-target species should be evaluated? By dividing biodiversity into functional categories that relate to specific risks (Andow & Hilbeck 2004; Snow *et al.* 2005), candidate species are rapidly identified (Birch *et al.* 2004). For example, 'non-target herbivores' is a functional category of concern because a transgenic crop may increase the risk of secondary pest problems, as has occurred from some insecticides.

Within functional categories, species can be identified that are most likely to cause a concern, either because of historical knowledge, their relative significance as, in this case, secondary pests, and their degree of association with the crop environment and the transgene product. Some of these concerns are particularly acute for the resource-limited rural poor in many developing countries, for whom a secondary pest outbreak could determine future livelihood.

Non-target and biodiversity risk assessments of transgenic plants continue to be improved. While indicator species continue to be used in many risk assessments, there is a trend towards assessing risks to non-target species that naturally occur in the local areas where transgenic crops will be planted. Because this approach corresponds to a case-specific risk assessment, it is likely to expand in the future as methods are improved and verified. Moreover, it will continue to be important to assess not only the effects of the transgenic plant itself, but also the effects associated with changes in agricultural practices and the impact of transgenic gene flow to wild relatives on the non-target community associated with those plants. Finally, although they may be difficult to imagine, effective methods for 'biodiversity' assessment need to be developed. These and other topics are discussed further in the final section of this manuscript.

## GENE FLOW AND ITS CONSEQUENCES

Gene flow is 'the incorporation of genes into the gene pool of one population from one or more other populations' (Futuyma 1998, p. 767). Gene flow between crops and wild species relatives has been occurring for thousands of years (Hancock *et al.* 1996; Ellstrand *et al.* 1999), but scientific attention on gene flow from crops to wild relatives and other crop populations is more recent, stimulated by concerns about the movement of transgenes (Snow & Morán-Palma 1997; Hall *et al.* 2000; Ellstrand 2001).

Regardless of whether transgenes are involved, the consequences of gene flow from crops can be problematic. Crops genes may replace wild genes (genetic assimilation: Ellstrand & Elam 1993; Levin *et al.* 1996; Wolf *et al.* 2001), reducing the genetic diversity of wild populations. Crop genes may also flow to other crop varieties or land races, contaminating the recipient seed pools. Whether this genetic contamination is called 'genetic pollution' or 'adventitious presence', it can have undesired consequences, reducing seed quality (Friesen *et al.* 2003), threatening food safety (NRC 2004a) and organic food production, or harming indigenous cultures [North American Free Trade Agreement–Commission for Environmental Cooperation (NAFTA–CEC) 2004]. If the resulting hybrids have lower fitness than their wild parents, the wild populations may shrink (demographic swamping: Levin *et al.* 1996; Wolf *et al.* 2001), threatening the survival of the wild population (Ellstrand & Elam 1993; Levin *et al.* 1996). Alternatively, if the resulting hybrids have a higher fitness than their wild parents, the hybrid may become invasive (Tiedje *et al.* 1989), replacing the wild population and other species in agricultural and natural areas. Gene flow from crops to wild relatives is implicated in the evolution of weediness in seven of the world's 13 most significant crops (Ellstrand *et al.* 1999).

Gene flow can occur by (a) seed and propagule dispersal (Crawley & Brown 1995), either by natural vectors (e.g. wind, water or animals) or by humans; (b) horizontal (non-sexual) transfer (which will not be addressed here; see Gebhard & Smalla 1998; Nielson *et al.* 1998; Bertolla & Simonet 1999; Ochman *et al.* 2000; Kay *et al.* 2002); or (c) pollen dispersal (Goodman & Newell 1985; Ellstrand 1988), which is primarily mediated by natural vectors such as wind and animals. Considerable research has been conducted recently on pollen dispersal (e.g. Ellstrand 2003; Pacini & Hesse 2004; Watrud *et al.* 2004; Yamamura 2004; Robledo-Arnuncio & Gil 2005), but as we argue below, under some conditions human-mediated seed dispersal may have a stronger influence on the risks associated with gene flow.

Prior to the 1980s, gene flow from crops was considered primarily as a seed production problem, because seed must be sufficiently pure to sell. During the late 1980s, it was widely believed that gene flow risks would not be significant for most transgenic plants (e.g. Regal 1986; Day 1987). In the 1980s and early 1990s, seed purity standards and management methods were used commonly for assessing risks of transgene flow [e.g. US Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) 1990], but as new information has accumulated, stricter standards have been used. In the early 1990s, the frequency of gene flow from crops was often underestimated. Although Ellstrand & Hoffman (1990) emphasized that crop genes could flow to recipient populations via many routes, it was not until 1997, that sufficient evidence had accumulated to allow Snow & Morán-Palma (1997) to suggest that when gene flow is possible, it is probable. By careful accumulation of many examples (Ellstrand *et al.* 1999, 2002; Ellstrand 2003) Ellstrand concluded that gene flow via seeds and pollen from crops was ubiquitous and evolutionarily significant for the recipient populations for nearly all of the world's important crops.

This change in scientific understanding is illustrated by oilseed rape (*Brassica napus* L.) and its wild relative *B. rapa* L. (Brassicaceae). Although it was widely appreciated that oilseed rape could produce viable hybrids with weedy *B. rapa*, early research emphasized the barriers to gene flow and the low likelihood of hybrid survival (Downey *et al.* 1980; Miller 1991; Crawley *et al.* 1993; Hails *et al.* 1997). Contrary to these expectations, Jørgensen & Andersen (1994) found that crop genes were transmitted readily from

oilseed rape to weedy *B. rapa*, and later, herbicide-tolerance genes, including transgenes, were found in weedy *B. rapa* (Mikkelsen *et al.* 1996; Hall *et al.* 2000). Management of gene flow initially used the isolation distances needed to meet hybrid seed purity standards [800 m for < 0.25% contamination; Association of Official Seed Certifying Agencies (AOSCA) 2004], but recent results suggest that this isolation distance may not be appropriate for managing gene flow risks of transgenic oilseed rape. Rieger *et al.* (2002) observed cross-pollination ≥ 3 km from a source, and Wilkinson *et al.* (2003) suggested that cross-pollination of *B. napus* may be inevitable in the UK. Finally, it was thought that feral *B. napus* would not persist more than a couple years and most transgenes would be disadvantageous to the recipient population. However, Simard *et al.* (2002) found that feral populations rapidly acquire secondary seed dormancy and can persist > 5 years, and Pessel *et al.* (2001) found that feral *B. napus* populations could persist for > 8 years. Moreover, Snow *et al.* (1999) showed that herbicide tolerance genes were not harmful in hybrids with weedy *B. rapa*, even when herbicides were not used. Gene flow from *B. napus* to *B. rapa* was found to be more extensive and hybrid persistence more likely than believed at the beginning of the 1990s.

Three main factors probably influence the rate and fate of gene flow from a crop to a recipient population: the dispersal kernel of the transgene (probability of movement vs. distance from the source), the frequency of introductions (single introduction vs. recurrent introduction), and the fitness of the transgene in the recipient population (Table 1). Considerable research is needed to relate the theory quantitatively to actual empirical cases. In this review, we will contrast two extreme dispersal kernels, global dispersal, where a propagule disperses to all locations with equal probability, and local dispersal, where a propagule can disperse only to the nearest-neighbour site. Whether dispersal in nature is global or local will depend on the spatial scale of concern and where the transgenic crop is planted within its growing range. If the transgenic crop were planted uniformly over the entire geographic range of the crop, then overlap in the localized dispersal kernels may make the dispersal process equivalent to global dispersal. If one is concerned about the effects of gene flow at spatial scales of 100–1000 km, which might be relevant when considering, for example, the risks to organic oilseed rape production in Denmark from commercial transgenic production outside Denmark, naturally vectored seed and pollen dispersal should be considered local dispersal. However, at spatial scales of 10–100 m, which might be relevant when considering the risks to organic oilseed rape production from neighbouring transgenic fields, both natural seed and pollen dispersal may be considered global.

**Table 1** Theoretical population genetic consequences of bilateral gene flow of a transgene following single vs. recurrent gene flow events. Assumptions include: (a) gene flow bilateral; (b) single dominant hemizygous or additive homozygous novel allele fixed in source population; (c) novel allele initially absent in recipient population; (d) mutation rate ≪ hybridization rate ≪ 100%

| Response is evaluated in recipient population | Global dispersal | | | | | | Nearest neighbour dispersal (hypothesized effects) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Single release (Huxel 1999; Wolf *et al.* 2001) | | | Recurrent gene flow (Haygood *et al.* 2004) | | | Single release | | | Recurrent gene flow | | |
| Relative fitness of novel allele | A (+) | N (0) | D (−) | A (+) | N (0) | D (−) | A (+) | N (0) | D (−) | A (+) | N (0) | D (−) |
| Initial change in genetic diversity | + | + | + | + | + | + | + | + | + | + | + | + |
| Long-term change in genetic diversity | 0 or − | + or 0 | 0 | 0 or − | 0 or − | +, 0 or − | 0 or − | + or 0 | 0 | 0 or − | + or 0 | + or 0 |
| Persistence of novel allele | Assimilation | None or assimilation | None or assimilation | Assimilation | Assimilation or co-exist | Co-exist | Assimilation | None or assimilation | None or assimilation | Assimilation | Coexist or assimilation | Coexist |
| Rate of change in novel allele frequency | Fast | Slow | Very slow | Fast | Fast or slow | Fast or slow | Fast | Slow | Very slow | Fast | Slow | Very slow |

A(+), transgene is selectively advantageous in recipient population; N(0), transgene is neutral; D(−), transgene is deleterious.

Recurrent and single introductions are the extreme cases of introduction frequency. Recurrent introductions occur each generation and the size of the introductions is similar each generation. Single introductions are never repeated. Actual patterns of introduction typically fall between these extremes. The effects of such intermediate introduction patterns will fall between the effects of the two extreme cases, but additional research is needed to quantify these relationships.

Genetic assimilation can be defined as fixation of the transgene in the recipient population. Under this definition, the rate of genetic assimilation will depend initially on deterministic migration-selection dynamics, but when the transgene nears fixation it will depend also on genetic drift, because even in large recipient populations genetic drift can be strong for rare alleles. If instead, genetic assimilation is defined as the transgene frequency > 0.8 in the recipient population, which we use in this review, then we can ignore the effects of genetic drift in large recipient populations and focus on the effects of migration and selection. This is helpful because the size of a large recipient population is defined implicitly by the migration rate. For example, a global pollen migration rate of 0.1 means that 0.1 of the pollen in the recipient population originates from the transgenic field. If the recipient population is of similar size or smaller than the transgenic population, this is realistically plausible; however, the recipient population could not be 100 times the size of the transgenic population because this would require more transgenic pollen that is produced.

The theory for global dispersal of transgenes is well-characterized. For global dispersal, transgenes that are selectively favoured in the recipient population can rapidly be assimilated (Huxel 1999; Wolf *et al.* 2001; Haygood *et al.* 2003) under both single (Huxel 1999; Wolf *et al.* 2001) and recurrent (Haygood *et al.* 2003) introductions. For example, under a modest migration rate (0.1) and relative fitness (0.05) a transgene can assimilate in 25 generations under recurrent introductions (Haygood *et al.* 2003). Neutral and deleterious transgenes can be assimilated irrespective of the frequency of introduction (Huxel 1999; Wolf *et al.* 2001; Haygood *et al.* 2003), but this is rare and slow for single introductions (Huxel 1999; Wolf *et al.* 2001) and potentially rapid for recurrent introductions (32 and 45 generations respectively with migration of 0.1 and relative fitness of either 0 or −0.05; Haygood *et al.* 2003). For deleterious transgenes, demographic swamping of the recipient population can occur (Haygood *et al.* 2003).

No theory has been published on localized dispersal of transgenes; however, work in related fields is likely relevant. It is likely that a selectively favoured transgene can assimilate rapidly under local dispersal (Table 1). Invasion models suggest that for many biologically reasonable conditions, a selectively favoured transgene might spread at a rate proportional to $(sD)^{1/2}$, where $s$ is the selective advantage and $D$ the variance of the dispersal kernel (Shigesada & Kawasaki 1997). For $s = 0.05$ and $D = 0.1$ km$^2$/generation, the transgene would spread at 70 m/generation. The rate of spread is determined by the selective advantage at the edge of the invasion front and the rate of dispersal from the front (Bramson 1983). Because neutral and deleterious transgenes will have no advantage at the edge of the invasion front, they cannot spread like an advantageous allele. For a single release, neutral or deleterious transgenes are likely to exhibit only transient persistence before they drift or are selected to extinction, similar to the outcome predicted under global dispersal. After a single introduction, the introduced transgene and its progeny are likely to be found near the point of introduction and unlikely to reach points farther away, compared with global dispersal. Consequently, transient persistence may be longer near the point of introduction but the probability of escape to far distances may be considerably lower than for global dispersal.

In contrast, for recurrent releases, the fate and rate of spread of neutral and deleterious transgenes are likely to differ for localized and global dispersal. Recurrent release acts as a migration pressure (Haygood *et al.* 2003), which is a powerful evolutionary force similar or greater in strength to selection. Under global dispersal migration pressure occurs simultaneously everywhere in the environment, and recurrent introduction can push both neutral and deleterious alleles to assimilate rapidly (Haygood *et al.* 2003). Under localized dispersal, however, migration pressure will decline with distance from the point of introduction, and at distances sufficiently far away, the fate of the transgene is determined by selection alone. Thus, neutral and deleterious alleles should be unable to assimilate unless the recipient population is small, in which case they could possibly drift to fixation. A neutral allele, however, could establish a stationary invasion front resulting from the migration pressure originating at the point of introduction. As migration pressure attenuates away from the point of introduction, genetic drift will predominate and the front may fluctuate randomly back and forth.

These theoretical investigations can provide an upper bound for the rate of genetic assimilation and demographic swamping. Theory suggests that genetic assimilation may be most rapid when the transgene is selectively advantageous in the recipient population, under recurrent introduction, and global dispersal. Whether a transgene is selectively advantageous is widely recognized as an empirical question that at present should be addressed on a case-by-case basis (Burke & Rieseberg 2003; Snow *et al.* 2003; Stewart *et al.* 2003; Ellstrand 2003). Recurrent introduction of a transgene is likely to be common for many transgenic crops, because they are annuals replenished each year with fresh seed.

However, if a transgene is planted at a small spatial scale, such as expected for many pharmaceutical crops, 'migration' pressure will be lower, and the rate of genetic assimilation will be correspondingly lower (Haygood *et al.* 2003).

Although strict global or local dispersal is unlikely to occur in natural settings, these extreme distributions still provide interesting qualitative insights. Because spatial scale determines whether dispersal is global or local, the relative significance of natural dispersal of pollen and seeds vs. human-mediated dispersal of seeds is likely to vary with spatial scale. For most plants, natural pollen and seed dispersal is likely to occur at spatial scales of < 10 km. Human-mediated seed dispersal, however, can vary considerably, from < 10 to > 1000 km. For example, in indigenous cultures, the scale of human-mediated seed dispersal should depend on the patterns of seed exchange within and between cultures. If seed exchange between cultures is low, then seed dispersal will be mainly localized at the spatial scale of the culture, but as exchange increases, seed dispersal may become global at that and larger spatial scales. For commercial commodity production, seed production and shipping may occur at spatial scales of > 1000 km, rendering seed dispersal global at nearly all practical spatial scales. Because genetic assimilation is faster under global dispersal than local dispersal, the risk of genetic assimilation in the maize landraces of indigenous peoples in Mexico would be predicted to depend in part on the spatial scale of seed exchange by these indigenous peoples, and the prevalence and rate of use of commercial, internationally distributed seed by nearby commercial maize producers. If seed exchange is local and use of commercial seed is very low, the risk of genetic assimilation may be low. However, if seed exchange is not local or use of commercial seed is common, the risk of genetic assimilation may be substantially higher, especially for selectively neutral or deleterious transgenes. The patterns of human-mediated seed dispersal may have greater influence on the risk and management of genetic assimilation than naturally vectored seed or pollen dispersal at all but the smallest spatial scales.

Managing gene flow to reduce its potential adverse effects has been proposed since the early 1970s (NRC 2004b). Physical methods, such as isolation by distance and plant destruction, have predominated because effective biological methods are still being developed (NRC 2004b). Some have suggested that management can be stratified between crops with low vs. high rates of gene flow (Raybould & Gray 1993; Stewart *et al.* 2003). However, others have suggested that gene flow rates will depend on ecological factors, such as distance, locality and season, and should not be considered a property of the crop alone (Ellstrand *et al.* 1989; NRC 2004b; Snow *et al.* 2005). Consequently, management should depend on both the crop species and the environment. Our theoretical analysis leads us to support this latter position.

Two recent events suggest that management of transgene flow may be difficult. In 2001, Quist & Chapela (2001) reported that transgenes were detected in traditional maize landraces in Oaxaca, Mexico, even though transgenic maize was not legally available in Mexico. Presently, illegal occurrences of transgenic crops have been reported from many countries, suggesting that seed movement will be difficult to manage. This followed closely on the 2000 occurrence of StarLink$^{TM}$ (Aventis Crop Science, Lyon, France) in the US food supply. Despite efforts to eliminate the StarLink$^{TM}$ gene, it can still be found in the US corn supply even though it has not been planted since 2000 (Marvier 2004). There are many biological methods suggested to manage the movement of plant transgenes, but none is ready for implementation, because failure rates and monitoring and remediation systems have not been fully considered (NRC 2004b). Moreover, the theory of gene flow management is very new. Haygood *et al.* (2004) have suggested that the time to escape of a transgene is a good indicator of effective management, because it clarifies how low leakage rates need to be to make escape times sufficiently long to be practically unimportant. They found that leakage rates $< 1 \times 10^{-3}$ could result in an appreciable probability of escape in < 10 generations. This suggests that gene flow management will require further development before it is ready for use. It may also be possible to manage risks after gene flow occurs. Ortiz-García *et al.* (2005) did not recover any transgenes in an extensive survey of Mexican land races during 2003 and 2004, suggesting that there may have been a decline in transgene contamination levels and opening up the possibility of post-gene flow risk management.

## RESISTANCE RISK AND MANAGEMENT

The evolution of pest resistance to pest control measures has been known for nearly 100 years, but it became a significant problem after World War II, when modern, intensive agricultural technologies proliferated, resulting in strong uniform selection over large areas. About 536 species of arthropods, 60 genera of plant pathogenic fungi, and 174 weed species have evolved resistance to pesticides (Eckert 1988; WeedScience.org 2003; Whalon *et al.* 2004), and resistance to Bt toxins has been documented in > 17 insect species (Tabashnik 1994; Huang *et al.* 1999).

Our understanding of resistance risk and management continues to evolve. Presently, none of the Bt crops now used has suffered a resistance failure despite widespread use. Whether this is because of effective resistance management or other factors is not generally known (Tabashnik *et al.* 2003). However, in some cases, such as Bt cotton in Australia, resistance management must have been crucial to avoiding failure, and in other cases, such as Bt cotton in Arizona, USA, other factors must also be important.

Despite some disagreement (reviewed in Tabashnik 1994), entomologists and weed scientists agree that resistance evolution is a risk for which some management intervention is desirable (NRC 1986). However, resistance management has been required only for transgenic insecticidal crops and not for transgenic herbicide tolerant crops, although this may change following recent reports of weed resistance (WeedScience.org 2003; Owen & Zelaya 2005). Interestingly, many virologists remain unconvinced that resistance will evolve to transgenic virus resistant crops (discussed by Tepfer 2002).

At the beginning of the 1990s, it had proven difficult to implement resistance management for any pesticide. By 2005, resistance management was required for transgenic insecticidal crops in nearly every country of the world, relying primarily on the high-dose/refuge strategy. This strategy delays the evolution of resistance by selecting against individuals heterozygous for resistance (Fig. 1, *RS*) and is defined by the three conditions necessary for its success: resistance is recessive, resistance is rare, and there is sufficient intermating between adults coming from refuges and Bt fields. The recessivity of resistance is related to the

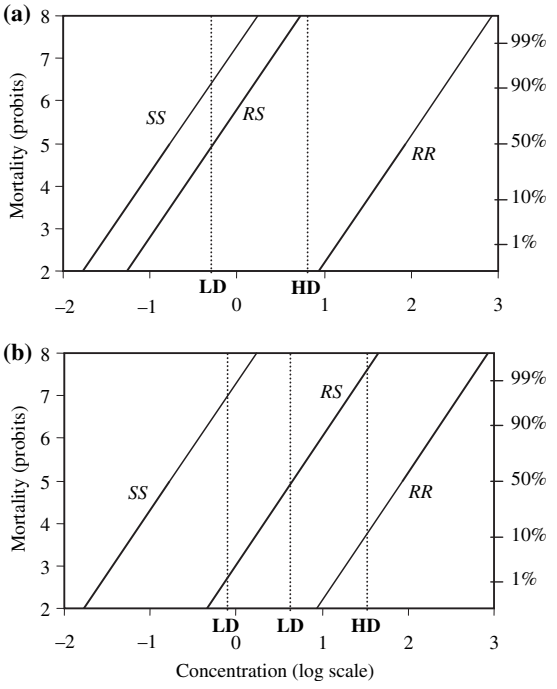


**Figure 1** High dose (HD) and low dose (LD) concentrations (dotted vertical lines) in relation to hypothetical mortality of *SS*, *SR*, and *RR* insect genotypes as a function of *Bt* crystal protein concentration (solid diagonal lines); *R* is a resistance allele and *S* is a susceptible allele. (a) *R* allele is physiologically recessive, so *SS* and *RS* mortality is similar. (b) *R* allele confers an intermediate, co-dominant level of physiological resistance, so *RS* mortality is about midway between *SS* and *RR* mortality.

'dose' of the insecticidal toxin in the transgenic crop. Dose is defined as a concentration of toxin in the crop plant in relation to the expression of the resistance phenotypes. For a high dose, the concentration of toxin must be sufficiently high that resistance is functionally recessive (Fig. 1, concentration **HD** or higher, Taylor & Georghiou 1979; Tabashnik & Croft 1982). Any other concentration is a low dose (Fig. 1, **LD**). A high dose is desired for resistance management because *RS* heterozygotes are killed by the Bt crop, which greatly reduces the rate of resistance evolution. For a low-dose crop, the *RS* heterozygotes have a selective advantage over the SS homozygotes, which accelerates resistance evolution when resistance is rare.

A refuge is an area of habitat within normal dispersal distance of the transgenic crop where the pest is not subjected to selection from the toxin and can produce a viable population (Ives & Andow 2002). Refuges reduce the selective advantage of the resistance allele and provide susceptible *SS* mates so that *RR* individuals in the Bt field produce *RS* offspring. A refuge must be within normal dispersal distance so that there is sufficient mixing and mating between individuals emerging in the refuge and Bt field (mating is not required to be random; Ives & Andow 2002). A refuge can be any habitat where the target species occurs, including the non-Bt crop, Bt crops expressing proteins that do not target the pest, other crops, and other non-crop plants, as long as the resistance allele does not have a selective advantage in these fields. If the refuge areas cannot support a viable population of susceptible homozygotes, then the population tends toward extinction, and there are insufficient susceptible individuals available to delay resistance evolution.

In the early 1990s, hopes for resistance management were reinvigorated (Gould 1994; Roush 1994), but it was not clear that a high-dose/refuge strategy would delay resistance enough with a reasonably sized refuge (Comins 1977). In a series of simple simulations based on Comins (1977) early work, Alstad & Andow (1995) showed that the high-dose/refuge strategy could delay resistance in European corn borer (*Ostrinia nubilalis* (Hübner), Lepidoptera, Crambidae) to Bt corn for > 30 years with a 50% non-Bt corn refuge. Subsequent research suggested that smaller refuges would also substantially delay resistance, proving that effective resistance management was possible theoretically (Gould 1998; Shelton *et al.* 2000).

The focus shifted to practicalities. Could a high-dose/refuge strategy be implemented? In the USA, this question was answered through a series of decisions made by the EPA. In early 1995, the EPA registered Bt potato, but no resistance management was required. By the end of that year, EPA issued conditional registrations and required the development of resistance management for all subsequent Bt crops (Matten *et al.* 1996). Conditional registrations were

used to motivate the development and implementation of a scientifically justified resistance management strategy while allowing commercial use in the interim.

Are the necessary conditions of the high-dose/refuge strategy fulfilled? Recessive resistance to Bt cotton (Cry1Ac) has been found in cotton budworm (*Heliothis virescens* (F.), Lepidoptera, Noctuidae; Gould *et al.* 1997; Gahan *et al.* 2001), cotton bollworm (*Helicoverpa armigera* (Hübner), Lep., Noctuidae; Akhurst *et al.* 2003) and pink bollworm (*Pectinophora gossypiella* (Saunders), Lep., Gelichiidae; Tabashnik *et al.* 2000), but no resistance has been found in cotton bollworm (*H. zea* (Boddie), Lep., Noctuidae). Resistance to Bt maize, which is the most widely grown Bt crop, has not been found in the widely distributed European corn borer (Bourguet *et al.* 2003) or in southwestern corn borer (*Diatraea grandiosella* (Dyar), Lep., Crambidae). Resistance has been found in the beetle *Chrysomela tremulae* F. (Coleoptera, Chrysomelidae) to Bt poplar (Génissel *et al.* 2003), but not in rice stemborer (*Scirpophaga incertulas* (Walker), Lep., Crambidae; Bentur *et al.* 2000) to Bt rice. Neither Bt poplar nor Bt rice is planted commercially at this time.

There have been several attempts to designate a high dose without reference to a resistance allele, because resistance has not been observed in some species. The EPA–SAP (1998) has suggested that 25 times the concentration of toxin at which 99% of the individuals in a susceptible population die [lethal concentration 99 ($LC_{99}$)] may function as a high dose. Caprio *et al.* (2000) argued that 50 times the $LC_{99}$ is better supported by the published data on insecticide resistance. These standards can be readily operationalized for transgenic Bt crops, but they may contribute a false sense of security about the validity of the high-dose assumption. However, a better operational definition has yet to be suggested.

The key issues in 1995 were how large a refuge was needed to delay resistance evolution, did the refuge need to be spatially structured relative to the Bt fields, and could the refuge be managed to limit pest losses? Aspects of some of these questions remain unresolved today. In Australia, Bt cotton did not provide a high-dose against the key pest, cotton bollworm *H. armigera*, and growers and regulators agreed to require 70% refuges to make the likelihood of resistance remote (Fitt 1997). In the USA, Bt cotton provided a high-dose against the key pest, cotton budworm *H. virescens*, but not against another important pest, *H. zea*, however, refuge requirements were set at 4% unsprayed or 20% sprayed refuge outside of the Bt field with minimal requirements of spatial structure (EPA 2001). In 2001, spatial structure requirements were added for Bt cotton along with other modifications (EPA 2001). These initial requirements and changes represented a compromise among various interests, although science also played a significant role.

Resistance management requirements for Bt corn developed with strong scientific input. Early in 1997, the USDA regional research committee NC-205 reviewed model results and information on the ecology of European corn borer and suggested to registrants and the EPA that a 20–25% refuge was needed near all Bt corn fields (Anon. 1998). Research results supporting this recommendation were published in the ensuing years (Onstad & Gould 1998; Hunt *et al.* 2001; Bourguet *et al.* 2003). One of the key results was a bioeconomic model suggesting that a 20% refuge would be nearly optimal for growers who consider the trade-off between the immediate costs of the refuge and delayed costs of resistance failures (Hurley *et al.* 2001). Canada required a 20% refuge within 0.5 miles (*c.* 800 m) of Bt corn in 1998, and during 1999 the USA required the same for the 2000 growing season and thereafter (EPA 2001).

Several scientific issues remain unresolved. Understanding the mechanisms of resistance is necessary to tailor resistance management to the particular system, but these are just beginning to be revealed for Bt crops (Gahan *et al.* 2001). In general, the details of adult movement and mating may play a key role in the evolution of resistance (Caprio 2001; Ives & Andow 2002), but estimating these movement (Carrière *et al.* 2004) and mating rates in the field is challenging. Farming practices, such as crop rotation (Peck & Ellner 1997), management of the refuge (Ives & Andow 2002; Onstad *et al.* 2002), and IPM approaches to pest management, may also affect the rates of resistance evolution. Significantly, a consensus for managing low-dose events (Fig. 1., *LD*) has yet to emerge, and scientific analysis of this problem is incomplete. While Australia implemented 70% refuges for one low-dose event, the USA has used 20% refuges for both high- and low-dose events. Finally, resistance management has been addressed primarily in developed countries (see Fitt *et al.* 2004 for an exception) where seed is purchased new each year. The effects of transgene flow and introgression into crop and non-crop refuges has not been thoroughly considered (parts of this issue have been addressed by Chilcutt & Tabashnik 2004).

Monitoring for the occurrence and frequency of resistance and methods to improve compliance to resistance management requirements among growers are areas of current research. The key monitoring problem is how to estimate resistance frequency when it is rare and recessive. As a rule of thumb, for the high-dose/refuge strategy to be effective, the frequency of resistance should be $< 0.001$ (Roush & Miller 1986). This implies that resistant phenotypes will be extremely rare, $< 1 \times 10^{-6}$, and that $> 10^6$ individuals from natural populations must be screened to estimate such low allele frequencies. This would appear a logistical nightmare, but one promising approach is the $F_2$ screen (Andow & Alstad 1998; Andow & Ives 2002; Stodola & Andow 2004). It is a genic screen and works by

inbreeding isofemale lines so that recessive phenotypes are expressed in the $F_2$ generation when they can be detected. If mated females are collected from natural populations, each carries four haplotypes (two of her own and two of her mate's) and only 250 female lines need to be screened instead of $10^6$ field-collected individuals. The $F_2$ screen has been used for several species (Bentur *et al.* 2000; Akhurst *et al.* 2003; Bourguet *et al.* 2003; Génissel *et al.* 2003). Other genetic and phenotypic methods have been used on some cotton pests (Gould *et al.* 1997; Tabashnik *et al.* 2000), but for recessive alleles phenotypic screens will have reduced sensitivity and higher cost than genic screens (Andow & Ives 2002). Compliance of farmers to resistance management requirements has been variable (Jaffe 2003; Agricultural Biotechnology Stewardship Technical Committee (ABSTC) 2005; Carrière *et al.* 2005; Goldberger *et al.* 2005). Improving compliance may require a combination of bioeconomic modelling, surveys of grower behaviour and motivations, and development of effective educational materials.

## TIERED RISK ASSESSMENT

We define a tier as a process within risk assessment that is initiated by a decision to collect information and data and ends with a decision either that risk can be and is assessed based on the available information and data, or that the risk cannot be assessed and additional information or data are needed (Fig. 2). All scientific authors have advocated that environmental risk assessment is tiered (Strandberg *et al.* 1998; Kjaer *et al.* 1999; Poppy 2000; Schuler *et al.* 2001; Dutton *et al.* 2002b, 2003b; Cowgill & Atkinson 2003; Hill & Sendashonga 2003). Its purpose varies among authors, but important factors include reducing the cost of environmental risk assessment, concentrating effort on the most serious risks, and providing a rapid assessment procedure for some transgenic plants. In general tiering is expected to allocate more effort and time to more serious risks and less to less serious risks.

Hill & Sendashonga (2003) provided a theoretical principle to relate the structure of the tiering process to its purpose. They suggested that tiering is structured to increase the level of detail of a risk assessment depending on the results of the previous tier, the nature of the decision to be supported, and the limitations of data available. None of the previous authors have incorporated data limitations into the tiering process. Some authors have suggested that tiers should be structured to determine that there is no significant risk at the end of each tier (e.g. Dutton *et al.* 2002b, 2003b). As we show below, this is an unnecessarily narrow view of the decision to be supported as it ignores the connection between risk assessment and risk management. Several authors have not distinguished between the information and data gathering process and the decision process (Poppy 2000; Schuler *et al.* 2001; Dutton *et al.* 2002b, 2003b), suggesting a scientifically prescribed process that ignores the needs of other stakeholders. Some of the proposed systems (e.g. Schuler *et al.* 2001; Cowgill & Atkinson 2003) do not have a decision point at the end of a tier to terminate the assessment process. However, elements of these proposals may be useful scientific procedures within a tier, such as the laboratory and small-scale field trials testing the leafhopper *Eupteryx aurata* L. on transgenic potato (Cowgill & Atkinson 2003), or the laboratory experiment evaluating parasitism rate by *Diaeretiella rapae* McIntosh on transgenic canola (Schuler *et al.* 2001).

Risk management should be possible at any tier in the assessment process. Under the Dutton *et al.* (2002b, 2003b

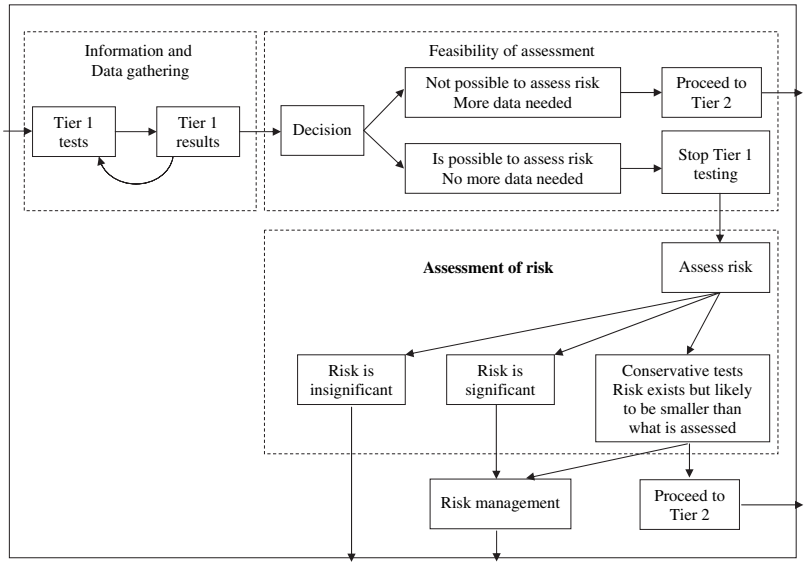


**Figure 2** Framework for tiered risk assessment showing the structure of a single tier. A decision is made to initiate the tier (left arrow), and information and data are gathered. Based on the information and data, a decision is made whether or not risk can be assessed and no additional data are needed. If so, the risk is assessed, and the transgenic crop is determined to have no significant risk (bottom left arrow), risks that require management (bottom right arrow), or higher tier evaluation is needed (right arrows).

model, risk management is reserved for the final tier, which is rarely done in practice. For example, the assessment of risk of resistance evolution to transgenic insecticidal crops is usually determined to be significant with minimal initial data (e.g. EPA 2001; Fitt *et al.* 2004), and risk analysis efforts shift quickly to risk management. Another example is provided by gene flow risks. Celis *et al.* (2004) conducted a series of experiments in the laboratory and field and determined that gene flow from transgenic potato to wild relatives in the central Andes would likely occur. This is the centre of biodiversity of potato, but Celis *et al.* (2004) suggested that gene flow risks can be managed using male-sterile cultivars, which would allow further evaluation of the efficacy of the transgenic potato.

Using Hill & Sendashonga (2003) as a guide, and incorporating elements of the risk analysis process (NRC 1983; EPA 1998) and unique elements from others (Strandberg *et al.* 1998; Kjaer *et al.* 1999; Poppy 2000; Schuler *et al.* 2001; Dutton *et al.* 2002b, 2003b; Cowgill & Atkinson 2003), we propose tiers (Fig. 2) in which each is structured to provide a definite outcome (proceed to the next tier of risk assessment, determination of no significant risk and stop risk analysis, determination of significant risk and begin risk management) without pre-supposing the outcome. Leaving and entering a tier are conscious decisions, and these decisions are based on an evaluation of the adequacy of the present information for conducting a risk assessment. Within a tier, we suggest that the information and data gathering processes be distinguished from the risk assessment and decision-making processes. This allows the intensiveness of the tiered process to be adjusted depending on the nature of the risk analysis and the decision to be supported. In addition, when an independent authority (with support from scientific experts) carries out the risk assessment, the assessment can be based on a larger set of data and hence be more accurate than if each single research group assessed the risk based on their data only. Finally, we suggest that data limitations should influence the decision to proceed to the next tier through the risk assessment at the end of each tier. The tiering process and the relationship among various steps within each tier remain controversial, and considerable work is needed before international standards and guidelines can gain scientific consensus.

## SOME FUTURE ISSUES

The 1980s brought the first scientific evaluations of potential environmental risks of transgenic organisms, including plants, and by the end of the decade there was a firm scientific consensus that environmental risks of transgenic organisms should be assessed on a case-by-case basis. The 1990s saw the development of a scientific consensus around the 'kinds' of risks that require evaluation for transgenic plants, and these have been classified as non-target and biodiversity risks, gene flow risks, and resistance risks. Although methodologies were available to assess all of these kinds of risks at the beginning of the 1990s, developments after 1990 have suggested important modifications to these methodologies. Indeed, as emphasized by Wolfenbarger & Phifer (2000), considerable scientific research is needed to meet the demands for scientific risk assessment. We address some of these key remaining demands here.

### Biological diversity risks

Risks to biological diversity involve the loss of biological diversity, namely the variability among living organisms including the ecological complexes of which they are part; this variability includes diversity within species, between species and of ecosystems [Convention on Biological Diversity (CBD) 1992]. A major challenge in determining the risk of transgenic plants on biological diversity is to separate natural variation from changes in the abundance of genotypes, species and ecosystem functions caused by the cultivation of transgenic plants. Because in general small-scale-field experiments are not sensitive enough to detect anything but large ecological effects (NRC 2002), assessment of risks to biological diversity will need to be conducted on a long-term, large-scale basis after commercialization of transgenic crops. This is the essential dilemma. We do not know what to look for, but to look for anything will be an expensive undertaking. NRC (2002) recommended a two-part approach to assess potential environmental impacts of transgenic crops that both take place after commercialization: first, trained observers should monitor areas with transgenic crops to detect unexpected effects. Based on their findings, scientific hypotheses will be generated and effects could be verified. The second recommendation is to carry out a long-term systematic monitoring for effects using relevant biological variables, and link this information to the patterns of cultivation of transgenic plants (NRC 2002). Inclusive procedures need to be developed to address the goals and methodologies for biodiversity risk assessment. Developing these procedures, identifying the relevant biological variables and securing the long-term funding necessary to conduct monitoring are significant challenges for the future.

### Potential risks of future transgenic crops

The commercialization of transgenic plants is likely to expand in future and will include a much larger variety of plants and traits than those that have been assessed to date.

These include plants with (1) increased stress tolerance (Farinha *et al.* 2004); (2) alterations to improve post-harvest processing such as a lower lignin content in trees; (3) modified food quality, novel products and increased nutrient content for human consumption; and (4) phytoremediation abilities (Bizily *et al.* 2000; Meagher 2000). New plant species will include turfgrass, pasture and tree species, and aquatic plants (Godfree *et al.* 2004; NRC 2004b; Van Frankenhuizen & Beardmore 2004; Watrud *et al.* 2004).

Many of these new transgenic species are more likely to establish feral populations and hybridize with wild relatives than the present commercial transgenic plants – maize, soybean, cotton and oilseed rape (Godfree *et al.* 2004; Van Frankenhuizen & Beardmore 2004; Watrud *et al.* 2004). As some of these newly transgenic species are already invasive in parts of their geographic range, if a transgene confers a fitness advantage, there is a risk that these species could become more invasive, invade new habitats and cause a loss in biodiversity and ecosystem functions.

Similarly, new traits such as stress-tolerance may increase competitive ability allowing the species to invade into natural habitats and/or replace natural or agricultural communities by expanding plantings into regions where the crop previously could not grow. For example, if aluminium-tolerant crops could be planted on a large scale in high aluminium, acidic soils (Herrera-Estrella 1999), such as savannas or cleared rainforests, this may reduce biodiversity or endanger or eliminate the original communities. This might be particularly devastating in savannas, such as the Brazilian cerrado, because they often sustain a high biodiversity (Scholes & Biggs 2005).

Nowadays, thousands of square kilometres of land and water have been polluted by human activities and transgenic phytoremediation – the use of transgenic plants to extract, sequester and/or detoxify pollutants such as heavy metals, radionuclides, and organic substances – is widely viewed as a promising method of reducing pollution in these areas (Bizily *et al.* 2000; Meagher 2000). While some of the future plants may have the ability to detoxify certain pollutants into non-toxic compounds, other plants will only be able to sequester pollutants into less toxic compounds or concentrate them for easier removal. Such plants may simply move the pollution problem from one location to another, and their environmental consequences should be assessed. For example, if a transgenic plant volatilized or transpired mercury from their tissues into the atmosphere (Bizily *et al.* 2000; Meagher 2000) over a large scale during an extended time, this may pose a serious hazard for human and animal health.

While the future benefits of transgenic plants may be great, it should be clear that the future will also bring increasingly complex challenges for environmental risk assessment. The present transgenic crops, such as insecticidal maize and herbicide tolerant soybean, have engendered considerable controversy as their environmental risks have been assessed, and although no large environmental risk has been documented, such results provide limited basis for assessing the risk of future transgenic crops. Instead, it will be most important that appropriate risk assessment methodologies are developed and implemented in regulatory systems that are suitably flexible to allow scientifically credible environmental risk assessment of these future transgenic plants.

This review has focused on the assessment of environmental risks of transgenic plants, but the potential environmental benefits also require rigorous scientific evaluation (Snow *et al.* 2005). Risk assessment methodologies will continue to evolve in the future, and additional research will be essential to ensure that this evolution is based on sound scientific information. Although change creates an uncertain regulatory environment, these changes should not preclude further development of useful transgenic plants. Risk assessment is always an imperfect science, and it should be developed so that there are many ways to manage this uncertainty. Despite its imperfections, however, it provides the main way for scientific methodologies and data to influence decisions to regulate commercialization of transgenic crops, and should be supported and improved by environmental scientists. Scientific understanding of the factors affecting environmental risk is still developing, and it is unlikely that controversies over environmental risks of genetically engineered organisms will be resolved in the near future. Thus, there is both the need and time for environmental scientists to have significant influence on the development of risk assessment methodologies for transgenic plants.

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