

# Genetically modified plants – the debate continues

Rosie S. Hails

Since the beginning of recombinant DNA technology, there has been a consensus among the scientific community that it is not the molecular methods themselves that are of immediate concern, but the phenotypes of the organisms they produce. (The catch phrase being 'it is the product not the process that should be examined'). The range of transgenics now in existence spans a wide variety of crops and constructs – here, examples are drawn from the most common construct groups: herbicide resistance, insect resistance and resistance to viral pathogens. Herbicide-resistant crops are already being grown in the USA and are the genetically modified organisms (GMOs) closest to being brought onto the market in the UK (Box 1). Insect- and virus-resistant crops have already been commercialized in the USA and China, but they are probably several years from possible commercialization in the UK (Boxes 2 and 3). Now is the time to weigh up the costs and benefits of this technology, and to consider the place of GMOs in an integrated pest management strategy.

One of the first issues that needs to be addressed is: what constitutes ecological risk? One broad definition is that an ecological threat might be posed if the presence of a plant containing a transgene results in a negative impact on the ecosystem, relative to the status quo. A comparison with the status quo is essential because modern agriculture has had considerable influence on the British countryside. The impact of a transgenic crop could be direct, through increased invasiveness, or indirect, via an alteration of agronomic practice. Ecological risks might be classified into three groups: (1) those concerning genome organization within the plant; (2) the escape of transgenes into wild relatives (the likelihood and the consequences); and (3) the impact on nontarget species in the wider ecosystem. As with many classifications, the edges are fuzzy; for example, the genomic location of a transgene can influence the rate of gene flow through hybridization<sup>1</sup>. Here, the focus is on the potential risks in groups 2 and 3.

## Gene flow and the consequences

The escape of transgenes into wild relatives (one of the concerns relevant to all GMOs) is a two-step process involving hybridization and establishment. The answer to the question 'will transgenic crops hybridize with wild relatives?' is almost certainly yes, although the frequency with which this occurs will depend upon the number and the closeness of wild relatives to the crop in question.

**The debate about the potential risks and benefits of genetically modified organisms (GMOs) has hit the headlines over the past few months. The polarization of much of the debate obscures what really constitutes ecological risk, and what methods we can apply to identify and quantify those risks. Ecological science has much to offer in this respect, including ecological theory, manipulative experiments, the application of molecular tools and the interpretation of observational data from conventional agriculture. In the current heated debate, it is perhaps belief in the scientific method, above all else, that needs to be promoted and discussed.**

Rosie Hails is at the NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, UK OX1 3SR (rha@wpo.nerc.ac.uk).

*How frequently does hybridization occur?*

Considering the first step of the escape process, hybridization, crops fall naturally into three groups<sup>2</sup>: (1) those for which there are no wild relatives present (e.g. maize in the UK); (2) those for which there are limited hybridization opportunities (e.g. oilseed rape, *Brassica napus*); and (3) those with very closely related wild relatives (e.g. sugar beet, *Beta vulgaris*, in the UK; sunflowers, *Helianthus annuus*, in the USA). Gene flow between crops and their closely related wild relatives has been leaving its fingerprints since modern agriculture began; for example, it has long been known that weed beet is a hybrid between sugar beet and wild beet<sup>3</sup>. More recently, a study of sunflower populations has

shown that molecular markers specific to cultivars are found frequently in wild sunflower populations<sup>4</sup>. Introgression (the spread of genes of one species into the gene pool of another species by hybridization) was found to be the most likely explanation for the patterns observed in the crop specific markers.

In the past few years, much attention has been on the group with limited hybridization opportunities, in which hybridization is known to be possible with a few species, but frequencies in the field were uncertain. Oilseed rape is known to hybridize with at least four British species through open pollination and up to five other species through manual pollination<sup>5</sup>, but how often does this actually occur in the field? One of the wild relatives, wild turnip (*B. rapa*), is often found close to oilseed rape fields. Screening of two such populations revealed that 0.4–1.5% of seeds were hybrid<sup>6</sup>. However, in other studies, hybridization rates can be anything between 10% and 90% depending upon the spatial distribution of the crop and the wild relative<sup>7,8</sup>. Isolated plants within oilseed rape fields are much more likely to hybridize with the crop than with individuals of populations adjacent to agricultural fields. This striking difference in hybridization rates illustrates the inherent variability often found in such ecological parameters in the field, and the difficulties associated with quantifying levels of risk<sup>9</sup>.

The fitness of first and second generation hybrids will also contribute to rates of introgression. First generation (F<sub>1</sub>) hybrids of *B. napus* and *B. rapa* are intermediate in fitness between the two parents, with some individuals close in fitness to their weedy parent<sup>10</sup>. However, second generation (F<sub>2</sub>) hybrids and backcrosses (F<sub>1</sub> × either parent) have a reduced fitness relative to both parents<sup>11</sup>, but with

considerable variance – some individuals being as fit as the parent plants. Although this drop in fitness by  $F_2$  hybrids will probably hinder the rate at which transgenes can establish and spread, it will not prevent introgression.

Therefore, it is the rate at which introgression is likely to occur that distinguishes crops with limited hybridization opportunities from those with closely related wild relatives (whereas introgression is not possible for crops with no wild relatives). But does introgression of transgenes *per se* pose an ecological risk? Neutral transgenes will not influence the population dynamics of the plant (by definition), however, a gene that is selectively neutral in one genetic background will not necessarily be so in another. It is possible that some transgenes will confer an ecological advantage to the plant (in particular, genes conferring pest resistance). These genes are the most likely to become established and therefore represent a greater potential risk. This leads to two avenues of enquiry: what are the fitness consequences to wild relatives of the introgression of transgenes, and are there ways in which gene flow can be contained?

*The consequences of hybridization: costs and benefits of transgenes*

Ecological theory suggests that herbicide resistance is costly to maintain in the absence of the herbicide, however, this hypothesis has been notoriously difficult to test because a comparison of conventional resistant and susceptible genotypes contains many confounding features (e.g. different genetic backgrounds and linkage effects)<sup>12</sup>. Only since the advent of genetic engineering, which has allowed much greater control over the genetic background, have these ecological questions been asked more precisely. Interestingly, the results have been equivocal. A study of herbicide resistance engineered into *Arabidopsis thaliana* demonstrated a 34% reduction in lifetime seed production as a consequence of carrying the herbicide resistance gene<sup>13</sup>. (However, reduced seed production does not necessarily translate to reduced fitness, because microsite availability, rather than fecundity, might be the limiting factor in natural habitats<sup>14</sup>.) By contrast, transgenic glufosinate tolerance introgressed from oilseed rape to weedy *B. rapa* was found to have negligible fitness costs associated with it<sup>15</sup>. Thus, these genes are not always as costly as might have been predicted, and might sometimes be effectively neutral.

However, insect and pathogen resistance are traits that could confer fitness advantages to plants in natural habitats. Species that can receive such transgenes through hybridization and introgression might increase in abundance to the detriment of others. Ecological release (the release of a population from the regulating influence of its natural enemies) has long been postulated as one of the major factors in the success of invasive exotic species<sup>16</sup>. Is it possible to make some assessment about which GM plants are likely to be invasive?

Theory provides us with the framework to estimate invasiveness, the simplest measure being the number of seeds produced per seed sown (the finite rate of increase)<sup>17</sup>. Manipulative experiments estimating changes in fitness are still relatively rare, particularly over more than one generation, although there are exceptions: oilseed rape resistant to insect herbivores does have increased fitness under insect selection pressure, but such fitness advantages are only apparent in the absence of plant competition<sup>18</sup>; and transgenic sugar beet engineered to be resistant to a viral pathogen showed higher produc-

**Box 1. Plants resistant to broad-spectrum herbicides**

**Potential benefits**

- Removes the need for pre-emergence spraying, thus these crops can be more easily integrated with zero and minimum tillage systems. This will reduce soil erosion, retain soil moisture and help conserve soil microfauna and flora<sup>40</sup>.
- The resistance genes most commonly used are for glyphosate or glufosinate resistance. These are less persistent than many herbicides, and so use of these might reduce toxic residues in groundwater<sup>41</sup>.
- A weed control strategy using herbicide resistant crops provides the opportunity to control certain herbicide-resistant biotypes; for example, black grass, *Alopecurus myosuroides*<sup>41</sup>.

**Potential risks**

- Introgression of transgenes into weedy relatives might cause herbicide resistant weeds. 'Gene stacking' would need to be prevented by management of herbicide use.
- Spray drift into field margins might have a direct impact on biodiversity and create opportunities for germination of herbicide tolerant volunteers by increasing microsite availability<sup>37</sup>.
- Broad-spectrum herbicides provide the opportunity for farmers to follow a 'squeaky-clean field' policy, which would have an adverse impact on farmland biodiversity through removal of food sources for beneficial species, for example, birds.

**Benefit or risk?**

It is uncertain whether the use of herbicide-tolerant crops will increase or decrease the diversity and quantity of herbicides used<sup>40</sup>. Diversity should be maintained to minimize selection pressures on weed populations, although quantity should be minimized.

tivity than the nontransgenic control under conditions of virus-infestation, but showed no apparent advantage under virus-free field conditions<sup>19</sup>. Clearly, there is a need for more experiments of this nature.

Although the term 'fitness' has a precise ecological meaning, it is not straightforward to ascertain if the altered fitness of a transgenic will result in it being invasive. Both absolute and relative estimates of fitness will vary with ecological conditions (as illustrated by the examples above), habitat and year<sup>20</sup>. In the face of such spatial and temporal heterogeneity, experimental manipulations are a means of artificially providing a wide range of biotic

**Box 2. Insect herbivore-resistant plants (with *Bt* transgenes)**

Transgenes that express proteins toxic to insects have been inserted. Pea lectins, for example, inhibit some insect digestive systems. The most common strategy is to use proteins of the bacterium *Bacillus thuringiensis* (*Bt*), which damage the insect midgut and cause massive water uptake.

**Potential benefits**

- Reduction in the use of toxic chemical insecticides<sup>42</sup>.
- Reduction in the impact on nontarget insects such as bees, parasitoids and predators.
- More effective control of the pest – expression of the insecticidal protein is normally constitutive (i.e. in all tissues at all times).

**Potential risks**

- Evolution of resistant pests would render not only the transgenic crop redundant, but also the use of *Bt* as a surface application. Selection could be rapid, especially when pest populations have high initial frequencies of resistance alleles<sup>43</sup>. Models indicate that the evolution of resistance could be managed by the provision of refugia and delivery of sufficiently high doses within the crop<sup>44</sup>.
- Impact on nontarget insects<sup>35,45-47</sup>.
- Ecological release: the question is whether resistance to herbivores alters the fitness of the plant. If ecological release occurred in a centre of diversity where the crop originated, then this could reduce the genetic pool available to breeders<sup>48</sup>.

**Box 3. Plants resistant to viral pathogens (with *cp* transgenes)**

Resistance to a plant virus can be conferred by a gene coding for the protein coat of the pathogen itself (a *cp* gene). Expression of low levels of the coat protein prevents disease symptoms from developing.

**Potential benefits**

- Reduction in the use of toxic chemicals to control the insect vectors of plant pathogens.
- Concomitant reduction in the impact on nontarget insects.
- More effective control of plant diseases – resistance can be expressed constitutively.
- More enduring resistance for the plant in the coevolutionary arms race.

**Potential risks**

- Ecological release: viral pathogens are known to have large impacts on plant survival and fecundity. We know little about their role in regulating plant populations in natural communities<sup>28</sup>.
- Transencapsulation: the protein coat of a plant virus includes recognition sites for insect vectors. Other plant viruses infecting the host might 'steal' the protein coat and so gain access to vectors and hence new plant hosts. Molecular alteration of recognition sites can prevent this<sup>28,29</sup>.
- Recombination: the mRNA of the *cp* gene might recombine with other plant viruses infecting the host, and thus give rise to novel competent viruses. It is unknown how often this happens in the field<sup>26,27,31</sup>.
- Synergism: other infecting viruses might interact with the transgenic product and produce disease symptoms of greater severity than pure infections<sup>27</sup>.

conditions and, as such, remain the best way to investigate potential invasiveness<sup>21</sup>. However, it is always possible to think of a set of ecological conditions that have not been tested – therefore, some consensus needs to be reached about how many, and which, experiments are required before a transgenic plant can be considered safe or unsafe.

*Can hybridization be contained?*

Hybridization can be limited, but not contained. Limitation can be brought about by the use of terminator technology or by integrating transgenes into chloroplasts. Terminator technology refers to any system (conventional or transgenic) in which progeny seed viability is genetically controlled. The use of male sterile lines has been used in conventional agriculture to prevent the escape of pollen, however, it is not universally applicable even with genetic modification techniques, and can be spontaneously reversible. 'Suicide genes' can be linked to pollen specific promoters, and thus might prevent pollen development or act later to prevent seed germination<sup>22</sup>. Thus, seed saved from a crop one year would not be viable for the next. Clearly, preventing the escape of transgenes through pollen is less desirable for those parts of the world dependent on farm-saved seed for subsequent crops. An alternative is to develop techniques to contain transgenes by integrating them into the chloroplast genome instead of the nucleus<sup>23</sup>. Plastid inheritance in most angiosperms is uniparental (maternal), thus this approach would prevent transgenes escaping via pollen. It is also possible to be more precise about exactly where in the chloroplast DNA the transgene is being inserted, thus reducing positional effects thought to be responsible for unstable transgene expression and for the occasional silencing of endogenous genes. The development of this technique has been greeted with optimism<sup>24</sup>, although there are caveats. The principal one is that transgene escape can still occur through hybrid formation with the crop being the pollen recipient rather than the donor. However, recent research on oilseed rape and wild *B. rapa* has illustrated that gene flow via this route is likely to be rare and sporadic<sup>25</sup>. Hence, this technique provides the possibility that gene flow can

be greatly reduced for some crops and constructs, if this would be of ecological benefit (and not to the detriment of the farmer).

*Gene flow via recombination*

Another potential path of gene flow is particularly pertinent to virus-resistant transgenic plants. One method of engineering resistance to a viral pathogen is to insert a gene derived from the pathogen itself, often the gene (or part of it) that codes for the protein coat of the viral pathogen (a *cp* gene). When this transgene is expressed, it interferes with the replication of an infecting virus, thus conferring tolerance.

One question is whether such pathogen-derived tolerance presents fundamentally different risks from other forms of pathogen resistance. The *cp* genes often confer more enduring resistance than other genes. This benefits the plant breeder because the rapid rates of coevolution of plant viruses result in new strains that circumvent many resistance genes. However, there are concerns that greatly increasing the frequency of *cp* genes in plant populations will accelerate the rate of evolution of natural virus populations<sup>26–28</sup>. Recombination is known to have been important in virus evolution<sup>29</sup>, and most viruses are a mosaic of differently rearranged genetic modules<sup>30</sup>. When closely related viruses infect a transgenic plant, they could recombine with the mRNA of the transgene, potentially producing novel, virulent genotypes. Viral genes often have unknown multifunctions and the *cp* gene might have unpredictable consequences in the new recombinant virus.

At present, any assessment of the potential of *cp* genes to accelerate the evolution of virulence among plant viruses remains speculative. Recombination between transcripts expressed by the plant and infecting viruses has been observed – although only in the laboratory. The resulting recombinants might be more or less virulent than their 'parents'<sup>31</sup>. There is still little idea of the frequency with which this occurs in the field. Plants expressing viral sequences that are then infected with another virus are in the equivalent position to plants that sustain multiple infections in the field<sup>32</sup>. Recent evidence of the prevalence and the distribution of viruses in wild cabbage has discovered that virus infections are common, and that >50% of all plants sampled have more than one virus<sup>33</sup>. Under these circumstances, recombination might have already had its maximum impact. Any potential risk will be minimized by only releasing transgenes from viruses that are already common in a particular crop and at a given locality. Recombinants between a viral transgene and any potentially co-infecting viruses should also be constructed experimentally, and tested for pathogenicity, virulence and host range<sup>34</sup>.

**The impact of GMOs on nontarget species**

The impact of GM crops on nontarget species might occur via several routes. Two examples are considered here: direct detrimental effects on nontarget insects (e.g. predators, parasitoids or insects of conservation value); and changes in farm management practice that impact on farmland biodiversity.

*The impact of GM crops on nontarget insect species*

A recent example to receive widespread publicity has been the case of the monarch butterfly (*Danaus plexippus*), a species of conservation value in the USA. Larvae feeding on milkweed (*Asclepias curassavica*) dusted with GM corn pollen [expressing *Bacillus thuringiensis* (*Bt*)] were found

to have reduced survival<sup>35</sup>. Because the host plant of this butterfly is found in the vicinity of corn fields, certain questions are raised. The relevant question from a risk assessment angle must be to compare these risks with those posed by the same crop, not genetically modified, but treated with *Bt* insecticide. How does mortality from GM pollen compare with that from insecticide spray drift? It is most desirable for control measures to have no impact on any nontarget species. However, where there is some harm, these results should be considered alongside the impact of conventional control measures in the same ecosystem.

#### *Changes in farm management practice that impact on farmland biodiversity*

Recently, it has been reported that by switching from spring to winter cereals (non-GM crops in both cases), the diversity of weed flora in Denmark has been significantly reduced<sup>36</sup> with potentially serious consequences for the ecology of farmland wildlife. In the UK, our agricultural environment is intimately intertwined with the countryside, and so any changes in farm practice could have significant knock-on effects for the wider ecosystem. For herbicide-resistant crops, ecological concerns centre on changes in the type and the frequency of herbicide usage. It is possible that spray drift of broad-spectrum herbicides into field margins could adversely affect those ecosystems, removing perennial species and allowing colonization by annuals (including volunteers from the GM crop itself)<sup>37</sup>. It is also possible that farmers will be able to remove weed species with much greater efficiency than in the past, and that this will reduce the abundance and diversity of farmland weeds and all the species associated with them. To address these questions precisely, the DETR (Dept of the Environment, Transport and the Regions in the UK) is launching a major research initiative to conduct farm-scale trials on herbicide-resistant crops. These issues are of the greatest urgency, given how close this class of GMOs is to being brought onto the UK market.

#### Conclusions

There is much debate about the costs and benefits of GMOs. The agricultural and wider ecosystems are so important to our well-being that it is vital that these issues are addressed. However, it is also important that the debate is a balanced one. Conventional agriculture already has a significant impact on the British countryside and any impact of GM crops should be considered in this context. It is important that new risks and irretrievable consequences are not introduced. However, the next question should be 'does this transgenic crop result in more or less environmental harm than current conventional alternatives?'

To date, there is no widely accepted framework as to what set of experiments would constitute an adequate risk assessment. The problem lies, in part, with the paucity of general principles that can be applied. It is known, for example, that gene flow will most certainly occur, but the consequences of this are uncertain. Ecologists are still in the position of considering each crop-construct combination on a case-by-case basis.

However, there has been some progress. If we consider the three classes of GMOs discussed, they occupy distinct positions on the risk assessment scale. Herbicide resistant transgenes are unlikely to confer fitness benefits. Concerns here centre around changes in farm management practice that might impact on biodiversity. Pest-resistant transgenes could confer a fitness advantage to crops or

(via introgression) to wild relatives. We know the kinds of experiment that are required to investigate when this is likely to happen (namely manipulative experiments to estimate invasibility). Finally, virus resistant plants represent a group of GMOs about which there are additional uncertainties concerning the conditions under which we can influence the evolution of novel competent and pathogenic genotypes. Future emphasis should be on the potential impact of a 'reservoir' of *cp* genes in the environment. Hence, it is now possible to broadly classify levels of potential risk represented by crops (in terms of gene flow<sup>2</sup>) and transgenes (in terms of potential impact<sup>38</sup>).

Much of the research into risk assessment is conducted in the laboratory, or in small-scale field experiments. The challenge of the future will be on how to extrapolate these results to scales relevant to commercial release. Evidence suggests that large pollen sources interact on a regional scale to increase estimates of gene flow<sup>39</sup> – illustrating that extrapolation is unlikely to be a linear process. Given such considerations, there might be no substitute for large-scale trials. As part of these trials, a rigorous scientific approach would be to compare any detrimental effects of a GM crop with those of the crop it is intended to replace. [The Royal Society (1998) Genetically modified plants for food use ([www.royalsoc.ac.uk/st\\_pol40.htm](http://www.royalsoc.ac.uk/st_pol40.htm))].

#### Acknowledgements

Thanks to John Beringer, Charles Godfray and two anonymous referees for suggesting improvements to the original manuscript.

#### References

- Rieseberg, C.H. *et al.* (1999) Introgression between the cultivated sunflower and a sympatric wild relative, *Helianthus petiolaris* (Asteraceae). *Int. J. Plant Sci.* 160, 102–108
- Raybould, A.F. and Gray, A.J. (1994) Will hybrids of genetically modified crops invade natural communities? *Trends Ecol. Evol.* 9, 85–89
- Boudry, P. *et al.* (1993) The origin and evolution of weed beet: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. *Theor. Appl. Genet.* 87, 471–478
- Linder, C.R. *et al.* (1998) Long-term introgression of crop genes into wild sunflower populations. *Theor. Appl. Genet.* 96, 339–347
- Scheffler, J.A. and Dale, P.J. (1994) Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Res.* 3, 263–278
- Scott, S.E. and Wilkinson, M.J. (1998) Transgene risk is low. *Nature* 393, 320
- Jørgensen, R.B. *et al.* (1996) Spontaneous hybridisation between oilseed rape (*Brassica napus*) and weedy relatives. *Acta Hort.* 407, 193–200
- Jørgensen, R.B. *et al.* (1997) Introgression of crop genes from oilseed rape (*Brassica napus*) to related wild species – an avenue for the escape of engineered genes. *Acta Hort.* 459, 211–217
- Gliddon, C.J. (1999) Gene flow and risk assessment. In *Gene Flow and Agriculture: Relevance for Transgenic Crops* (Lutman, P.J.W., ed.), pp. 49–56, British Crop Protection Council
- Hauser, T.P. *et al.* (1998) Fitness of F<sub>1</sub> hybrids between weedy *B. rapa* and oilseed rape (*B. napus*). *Heredity* 81, 429–435
- Hauser, T.P. *et al.* (1998) Fitness of backcross and F<sub>2</sub> hybrids between weedy *Brassica rapa* and oilseed rape (*B. napus*). *Heredity* 81, 436–443
- Bergelson, J. and Purrington, C.B. (1996) Surveying the costs of resistance in plants. *Am. Nat.* 148, 536–558
- Bergelson, J. *et al.* (1996) Costs of resistance: a test using transgenic *Arabidopsis thaliana*. *Proc. R. Soc. London Ser. B* 263, 1659–1663
- Bergelson, J. (1994) Changes in fecundity do not predict invasiveness. *Ecology* 75, 249–252
- Snow, A.A. and Jørgensen, R.B. (1999) Fitness costs associated with transgenic glufosinate tolerance introgressed from *Brassica napus* ssp. *oleifera* (oilseed rape) into weedy *Brassica rapa*. In *Gene Flow and Agriculture: Relevance for Transgenic Crops* (Lutman, P.J.W., ed.), pp. 137–142, British Crop Protection Council

- 16 Dobson, A.P. and May, R.M. (1986) Patterns of invasions by pathogens and parasites. In *Ecology of Biological Invasions of North America and Hawaii* (Mooney, H.A. and Drake, J.A., eds), pp 58–77, Springer Verlag
- 17 Crawley, M.J. *et al.* (1993) Ecology of transgenic oilseed rape in natural habitats. *Nature* 363, 620–623
- 18 Stewart, C.N. *et al.* (1997) Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. *Mol. Ecol.* 6, 773–779
- 19 Bartsch, D. *et al.* (1996) Competitiveness of transgenic sugar beet resistant to beet necrotic yellow vein virus and potential impact on wild beet populations. *Mol. Ecol.* 5, 199–205
- 20 Kareiva, P. *et al.* (1996) Can we use experiments and models in predicting the invasiveness of genetically engineered organisms. *Ecology* 77, 1670–1675
- 21 Mack, R.N. (1996) Predicting the identity and fate of plant invaders: emergent and emerging approaches. *Biol. Conserv.* 78, 107–121
- 22 Keeler, K.H. *et al.* (1996) Movement of crop transgenes into wild plants. In *Herbicide-resistant Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects* (Duke, S.O., ed.), pp. 303–330, CRC Lewis
- 23 Daniell, H. *et al.* (1998) Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nat. Biotechnol.* 16, 345–348
- 24 Gray, A.J. and Raybould, A.F. (1998) Reducing transgene escape routes. *Nature* 392, 653–654
- 25 Scott, S.E. and Wilkinson, M.J. (1999) Low probability of chloroplast movement from oilseed rape (*Brassica napus*) into wild *Brassica rapa*. *Nat. Biotechnol.* 17, 390–393
- 26 Tepfer, M. (1993) Viral genes and transgenic plants. *Biotechnology* 11, 1125–1132
- 27 Robinson, D.J. (1996) Environmental risk assessment of releases of transgenic plants containing virus-derived inserts. *Transgenic Res.* 5, 359–362
- 28 Cooper, J.I. (1997) Might transgenes conferring virus resistance harm the environment? In *Commercialization of Transgenic Crops: Risk, Benefit and Trade Considerations* (McLean, G.D. *et al.*, eds), pp. 115–124, Bureau of Resource Sciences
- 29 Keese, P. and Gibbs, A. (1993) Plant viruses: master explorers of evolutionary space. *Curr. Opin. Genet. Dev.* 3, 873–877
- 30 DeJong, W. and Ahlquist, P. (1992) A hybrid plant RNA virus made by transferring the noncapsid movement protein from a rod-shaped to a icosahedral virus is competent for systemic infection. *Proc. Natl. Acad. Sci. U. S. A.* 89, 6808–6812
- 31 Salanki, K. *et al.* (1997) The biological properties of recombinant CMV strains relevant to the biosafety of transgenic virus resistant plants. *The 3rd JIRCAS Symposium: The 4th International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, JIRCAS International Symposium Series 5*, 227–232
- 32 de Zoeten, G.A. (1991) Risk assessment: do we let history repeat itself? *Phytopathology* 81, 585–586
- 33 Raybould, A.F. *et al.* (1999) The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. *New Phytol.* 141, 265–275
- 34 Teycheney, P.Y. and Tepfer, M. (1999) Gene flow from virus-resistant transgenic crops to wild relatives or to infecting viruses. In *Gene Flow and Agriculture: Relevance for Transgenic Crops* (Lutman, P.J.W., ed.), pp. 191–196, British Crop Protection Council
- 35 Losey, J.E. *et al.* (1999) Transgenic pollen harms monarch larvae. *Nature* 399, 214
- 36 Hald, A.B. (1999) The impact of changing the season in which cereals are sown on the diversity of the weed flora in rotational fields in Denmark. *J. Appl. Ecol.* 36, 24–32
- 37 Sweet, J.B. *et al.* (1997) The impact of releases of genetically modified herbicide tolerant oilseed rape in the UK. In *Proceedings of the 1997 Brighton Crop Protection Conference, Brighton, UK, 17–20 November 1997* 1, 291–302, British Crop Protection Council
- 38 Sweet, J.B. *et al.* (1999) Assessing the impact and consequences of the release and commercialisation of genetically modified crops. In *Gene Flow and Agriculture: Relevance for Transgenic Crops* (Lutman, P.J.W., ed.), pp. 241–246, British Crop Protection Council
- 39 Squire, G.R. *et al.* (1999) Gene flow at the landscape level. In *Gene Flow and Agriculture: Relevance for Transgenic Crops* (Lutman, P.J.W., ed.), pp. 57–64, British Crop Protection Council
- 40 Marshall, G. (1998) Herbicide tolerant crops – real farmer opportunity or potential environmental problem? *Pestic. Sci.* 52, 394–402
- 41 Duke, S.O. (1998) Herbicide resistant crops – their impact on weed science. *J. Weed Sci. Technol.* 43, 94–100
- 42 Peferoen, M. (1997) Progress and prospects for field use of *Bt* genes in crops. *Trends Biotechnol.* 15, 173–177
- 43 Gould, F. *et al.* (1997) Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3519–3523
- 44 Mallet, J. and Porter, P. (1992) Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? *Proc. R. Soc. London Ser. B* 250, 165–169
- 45 Hilbeck, A. *et al.* (1998) Effects of transgenic *Bacillus thuringiensis* corn fed prey on the mortality and development time of immature *Chrysoperia carnea* (Neuroptera, Chrysopidae). *Environ. Entomol.* 17, 480–487
- 46 Pilcher, C.D. *et al.* (1997) Preimaginal development, survival and field abundance of insect predators on transgenic *Bacillus thuringiensis* com. *Environ. Entomol.* 26, 446–454
- 47 Yu, L. *et al.* (1997) Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (Collembola: Isotomidae) and *Oppia nitens* (Acari: Orbatidae). *J. Econ. Entomol.* 90, 113–118
- 48 Kling, R.A. (1996) Could transgenic supercrops one day breed superweeds? *Science* 274, 180–181

## Erratum

Lee, C.E. and Bell, M.A. *Trends Ecol. Evol.* 14, 449

The first sentence of the third paragraph should read: ‘Strayer’s statements highlight the problem of using species distributions to infer pathways of freshwater invasions, especially when systematic relationships among populations are uncertain.’

### and not

‘Strayer highlights the problem...’

The latter implies that Strayer was making the point, whereas his letter actually exemplifies the problem.

**We apologize to the authors and our readers for changing the meaning.**

PII:S0169-5347(99)01790-5

## TREE Subscriptions

Personal subscribers – remember to renew your subscription now to ensure an unbroken supply of *TREE* into 2000.

See p. IV for details of our worldwide subscription services, or contact our customer services by fax, telephone or e-mail.

Tel: +44 1444 475650

Fax: +44 1444 445423

E-mail: [ct.subs@bi.co.uk](mailto:ct.subs@bi.co.uk)