#### Alan Gray

is a research scientist with a specialist interest in ecology and genetics. Professor Gray was a member of the Advisory Committee on Releases to the Environment (ACRE) from 1994 and its Chairman from 1999 until 2003. He is a member of the UK Government GM Science Review Panel.

Keywords: GM crops, invasiveness, cross-pollination, non-target effects, farmland biodiversity, commercial release

Professor Alan Gray Centre for Ecology and Hydrology, CEH Dorset, Winfrith Technology Centre, Dorchester, Dorset DT2 8ZD, UK

Tel: +44 (0) 1305 213500 Fax: +44 (0) 1305 213600 E-mail: AJG@ceh.ac.uk

# Genetically modified crops: Broader environmental issues

#### Alan Gray

Date received (in revised form): 3rd December, 2003

#### Abstract

Looking back over the five years since a similar review of the field, this paper considers some of the research designed to address those uncertainties that, in part, led to the delay in the commercial-scale release of genetically modified (GM) crops in the UK. This research has included studies of the impact of transgenes on crop biology and invasiveness, the frequency and consequences of hybridisation between crops and their wild relatives and, in two costly large-scale investigations in the USA and UK respectively, attempts to assess the environmental impact of GM crops grown on a commercial scale. The first, on the effects of Bt corn on the Monarch butterfly, has important lessons for the risk assessment process. The second, farm-scale evaluations of GM herbicide-tolerant crops, should provide a blueprint for the management of the crops within an agricultural landscape delivering both food and biodiversity.

## INTRODUCTION

A review of this topic published five years ago<sup>1</sup> provides both an opportunity to chart progress since then and a framework for this paper. In 1999, in response to pressure from environmental organisations and by agreement between industry and government, the commercial release of genetically modified (GM) crops had been put on hold and the UK farm-scale trials of GM herbicide-tolerant oilseed rape, beet and forage maize had begun. The first results from those trials have recently been published<sup>2</sup> and a panel of scientists appointed by government as part of the public debate on GM food crops in the UK has produced its first report.<sup>3</sup> In addition, research targeted at resolving some of the ecological uncertainties is now appearing in the scientific literature.

This paper reviews these developments and, dividing the potential environmental impacts of GM crops (as before<sup>1</sup>) into (i) the direct impacts of transgene 'escape' and (ii) the less direct impacts resulting from large-scale cultivation, assesses the extent to which the debate on environmental issues has moved on. What has been resolved? What uncertainties remain? Do the uncertainties constitute a reason for continuing to delay the commercial release of those crops currently close to market but held up in a regulatory hiatus?

# DIRECT IMPACTS OF GENE 'ESCAPE'

### Volunteers and ferals

Central to the assessment of potential risks from GM crop plants is an understanding of the effect of the inserted DNA on the biology of the plant and, in particular, some measure of whether the plant is likely to be more persistent or invasive. In the context of 'volunteers' (plants derived from spilled seed or tubers, usually in the following year's crop) and feral populations, which establish in frequently disturbed peri-agricultural habitats such as headlands and farm tracks, there have been no reports that any of the GM crops trialled or released commercially around the world have shown increased persistence. The major potential agronomic problem identified to date is the accumulation in single genotypes of more than one transgene (gene stacking) conferring resistance to more than one herbicide on cultivated plants when used

GM crops: Broader environmental issues

as part of arable crop management. This has already occurred in herbicide-tolerant canola (oilseed rape) in Canada<sup>4</sup> where the creation of multiple herbicide-tolerant volunteers has led to changes in weed control practices.<sup>5</sup>

Invasion of the wider environment outside agriculture, of semi-natural habitats, is generally much more difficult for such arable crop plants. A recent longterm study<sup>6</sup> has shown that a range of transgenic crops (glufosinate-resistant oilseed rape and maize, glyphosateresistant sugar beet and potato containing insecticidal proteins) were no more persistent or invasive, in a range of 12 habitats over ten years, than their conventional counterparts. This, and earlier research. has confirmed that invasion of semi-natural habitats is unlikely in crop plants where 'weedy' traits have been selected against during many, often thousands, of generations of domestication.

Although they are no more likely to invade semi-natural habitats than conventional crops, the advent of GM crops presents the opportunity to investigate the effect of single genes (and in time possibly suites of genes) on the fitness of crop plants outside cultivation. Which genes are likely to overcome the disadvantages imposed by traits of domestication? Some candidate genes have been investigated, notably in the relatively weedy, relatively recently domesticated, oilseed rape. For example the seeds of oilseed rape with modified oil content (high stearate) appear to be more long-lived in the soil seed bank.<sup>7</sup> Importantly, this attribute was not sufficient to make the high stearate genotype more invasive, since it appeared to have reduced seedling vigour.8 Similarly, in another experiment, the increased seed production of oilseed rape populations protected against insect herbivores was offset by high levels of seedling mortality from a range of other causes.<sup>9</sup> Thus it is important to investigate the impact of a novel gene on the entire life history of the plant and to measure its

effect on changes in population growth rates. A combined experimental and modelling approach<sup>10</sup> is likely to be required to predict whether any future fitness-associated genes (eg drought tolerance, disease resistance) really do increase the invasive potential of the crop in the wild.

## Cross-pollination and hybridisation with wild relatives

A more likely route for the 'escape' of transgenes (and one that is also extremely uncertain) is via hybridisation and gene flow to populations of wild relatives. Last year saw the first evidence of transgene escape to a wild relative from a commercially released GM crop – resulting from hybridisation between herbicide-tolerant oilseed rape and wild turnip (*Brassica rapa*) in Canada.<sup>11</sup>

Again it is important to draw a distinction between cross-pollination within the agricultural environment and that within the wider semi-natural environment. In agriculture, crosspollination may occur between GM and non-GM crops and feral populations (intraspecific) or between crops and wild relatives that are agricultural (interspecific) weeds. The first event, while critically dependent on the crop species, is, in outbreeding species such as forage grasses and partly outcrossing species such as oilseed rape, more or less inevitable. This fact has driven the debate about the coexistence of GM crops and other forms of agriculture, especially the organic sector. In particular, the need to maintain both varietal purity and consumer choice will require a clear policy regarding GM and non-GM crop separation distances and agronomic practices. Interspecific hybridisation within the agricultural environment, between crops and related weeds, has the potential to create problems where, as in the case of the transfer of herbicide tolerance to a weedy Brassica, the transgene concerned is part of a weed control strategy. This is not a new problem, but the evolution of wild turnip

Last year saw the first evidence of transgene escape to a wild relative from a commercially released GM crop

The need to maintain both varietal purity and consumer choice will require a clear policy on growing GM and non-GM crops

It is important to investigate the impact of a novel gene on the entire life history of the plant

#### These studies reaffirm the need to take a caseby-case approach

Only those genes with a selective advantage are likely to spread

It is appropriate to adopt a Darwinian paradigm in attempting to predict the consequences of gene flow or weed beet resistant to broad spectrum herbicides is a prospect to be avoided.

Studies published during the past five years have indicated that hybridisation rates and degrees of introgression may be very different between farmed and seminatural habitats. In both oilseed rape and sugar beet crops, hybridisation rates between crop and weedy relatives can be high (depending on a range of factors). Furthermore, in at least two well-studied situations, organic farmers' fields in Denmark where oilseed rape and wild turnip co-occur, and in sugar beet fields in northern France, there is clear molecular evidence of introgression (the incorporation of the gene - in these cases not transgenes - into the genome of the wild species by several generations of hybridisation and backcrossing).<sup>12,13</sup> In contrast, hybridisation rates to the naturalised form of B. rapa (which occurs on river and canal banks), which depend mostly on the wild populations and the crop growing closely together (sympatry), are relatively low.<sup>14</sup> Although no evidence of introgression from crop to the naturalised form of B. rapa has been published, genetic analysis of a wild relative of sugar beet, sea beet (Beta *maritima*), suggests that even in sympatric populations (populations occurring in the same area and theoretically able to hybridise), introgression from crop to wild relative is limited or absent (although the transfer of weed seed to wild populations of sea beet has been demonstrated<sup>15</sup>).

The picture emerging from these, and other, studies provides strong support for the view that it is appropriate to adopt a Darwinian paradigm in attempting to predict the extent, and more importantly the consequences, of gene flow. Notwithstanding its importance in plant species evolution, the many barriers to introgression, particularly between crops and wild relatives, will be counteracted only by strong selection pressures in the habitat of the wild plant. Herbicideresistant volunteers and weeds may flourish in areas where the herbicide is continuously applied, but are likely to be at a disadvantage in semi-natural habitats. These studies also reaffirm the need to take a case-by-case approach in assessing the impact on fitness, and hence potential environment impact of each transgene, or transgene combination, in each crop species and wild relative.

# Horizontal gene transfer

An issue that has come to the fore in recent years has been the concern that DNA from GM crops might transfer to soil microbes by horizontal gene transfer (the transfer of genetic material between organisms with distant genetic relationships in such a way that the genes become heritable in the recipient<sup>3</sup>). While there are no reports that soil bacteria have acquired genes from crop plants in the field there is a small possibility that this could happen -apossibility that may be higher for the current transgenes as they contain DNA derived from bacteria. As with vertical (sexual) gene flow only those genes that confer a selective advantage on the recipient organism are likely to spread and have an impact on microbial communities.

## LESS DIRECT IMPACTS OF GM CROPS Evolution of resistance and non-target effects

Five years ago the concerns that GM crops engineered to constitutively express insecticidal or antifeedant proteins might lead to the rapid evolution of resistance in the target pest, or have a range of adverse effects on beneficial non-target insects, were based largely on laboratory experiments. Since then, greater experience with managing insect-resistant crops, especially those expressing C<sub>ry</sub> proteins derived from the soil bacterium *Bacillus thuringiensis* (Bt), and, among other research, an extensive study of the Monarch butterfly, have helped to illuminate these problems.

Fears that resistance to Bt toxins might evolve rapidly in GM crops have not been realised. There have been no reports of breakdown of resistance in the field in crops expressing Bt genes, some of which have been cultivated since 1996 (mainly maize and cotton). Experience suggests that, as in non-GM cultivars, breakdown of resistance will happen (it has been observed in some target pests in the laboratory), but for the moment Bt appears to be offering a prolonged resistance mechanism against a specific group of pests. The delay of breakdown is likely to have been facilitated by the widespread adoption of high dose/refugia strategies in which pest-susceptible varieties are grown alongside the Bt crops. The proportion of the area planted as a refuge varies, depending on the crop and the pest (eg 20 per cent of the US corn belt, 30 per cent of Australian cotton), and other management strategies, including the incorporation of more than one gene, are being tried to further delay breakdown of resistance. Transgenes conferring resistance to various viral diseases in a range of crops (eg squash, papaya, rice, potato, tomato), and one conferring resistance to bacterial leaf blight in rice, have all provided efficient protection to date. These examples suggest that, although target pests are expected to evolve resistance to GM crops, the durability of the crop's resistance mechanism is no less than that of conventionally bred crops.

A series of laboratory studies described in the 1999 paper<sup>1</sup> have alerted us to the possible implications for the wider environment of growing GM pestresistant crops - and particularly the potential for harm to non-target, even beneficial, species. Among the groups of species that could be affected are those that feed on pest species (the examples given were ladybirds feeding on aphids and lacewings feeding on corn borers) or their parasites. Such studies of tritrophic interactions under controlled laboratory conditions have continued, providing an assessment of the potential impacts of insecticidal GM plants on other invertebrates (see GM Science Review

Panel<sup>3</sup> for an updated review). They essentially identify harm. However, in order to assess the actual environmental impact, or risk, it is necessary to have some measure of the exposure of the nontarget organism to that harm (risk = exposure  $\times$  harm). Unfortunately to date there have been relatively few field studies. An exception is the study of the Monarch butterfly (*Danaus plexippus*).

In 1999 a note in *Nature* appeared with the headline 'Transgenic pollen harms Monarch larvae',<sup>16</sup> sparking off a huge public outcry. The caterpillars of this iconic species of butterfly, when fed milkweed leaves dusted with pollen from Bt corn, suffered significantly increased mortality and reduced body mass compared with those fed on leaves with non-Bt pollen. A series of extensive follow-up studies, aimed at measuring the effect of Bt corn pollen on Monarchs under typical cultivation conditions, was eventually published in a special collection of the Proceedings of the National Academy of Sciences in 2001 (eg Sears et al.<sup>17</sup>). These studies demonstrated that in the field, pollen densities rarely reach levels where they could begin to have an effect (although one early Bt corn variety, Event 176, had adverse effects at low density), there is a limited overlap between the time when Bt pollen is shed and the young larvae are present, and only a proportion of Monarch caterpillars feed on milkweeds in cornfields. Furthermore the causes of mortality in the butterfly included conventional insecticides. Taken together the research demonstrates that Bt corn poses a negligible risk to populations of the Monarch butterfly. The Monarch case study<sup>4</sup> has rapidly become a classic example of risk assessment, illustrating that it is imperative to measure exposure in the field to any harm identified in the laboratory.

Despite this classic study, the broader, secondary, impacts on non-target species are likely to be difficult to resolve in detail in advance of the commercial-scale release of a specific GM crop. The experience in developing laboratory-based protocols as

The durability of GM crops' resistance mechanism is no less than that of conventionally bred crops

It is imperative to measure exposure in the field to any harm identified in the laboratory

If introduced in a widespread and unmanaged way, GMHT oilseed rape and beet could lead to further declines in farmland biodiversity – but GMHT forage maize could be broadly beneficial the first tier of a risk assessment combined with increased understanding of arable ecosystems will prove invaluable, both in designing experiments to examine particular hypothesised effects and also in designing monitoring schemes to test the assumptions of the risk assessment.

# Farmland biodiversity

The much publicised and eagerly awaited results of the UK's government-funded farm-scale evaluations (FSEs) of GM herbicide-tolerant (GMHT) crops<sup>18</sup> have recently, like the Monarch butterfly study, been published in a special issue of a peer-reviewed scientific journal (in this case Philosophical Transactions of the Royal Society B, Vol. 358, No. 1439<sup>2</sup>). Eight papers describe in detail the results of the four year study of spring-sown oilseed rape, beet and maize (winter-sown oilseed rape results are to follow) involving more than 200 fields distributed throughout Great Britain. Each field was divided, and half sown with the GM crop and half with its conventional equivalent, the major objective of the experiment being to see if growing GM crops tolerant to broad-spectrum herbicides (glufosinateresistant oilseed rape and maize, and glyphosate-resistant beet) affected the abundance and diversity of farmland wildlife compared with conventional crops. Thus the study was one of comparative herbicide management regimes, not one comparing GM per se with non-GM. The rationale behind the FSEs has been discussed elsewhere.<sup>1,18,19</sup>

The results, as expected, are different for each crop but are clear and consistent. They show that the herbicide management regime had a significant effect on the abundance of infield weeds and a range of invertebrate species in all three crops irrespective of inter-annual or regional variation (the latter perhaps an unexpected result). Broadly speaking over the lifetime of the crop GMHT oilseed rape and beet fields had fewer weeds, produced fewer seeds and had fewer insects of those species dependent on weeds (eg butterflies, true bugs, seedeating ground beetles) than did fields of their conventional counterparts. On the other hand, springtails, and some of their predators such as a species of ground beetle, were more abundant in the GMHT beet and oilseed rape than in the conventional crops (probably because springtails feed on decomposing weeds which were more abundant in the GMHT crops). In contrast, fields of GMHT forage maize produced three times the weed density and biomass of conventional forage maize fields. GMHT maize also supported more butterflies and bees, although the numbers in maize fields generally are low.

The consistency of results across different farms and regions and the clear patterns linking different trophic groups give some confidence that the results can be scaled-up, and that predictive models can be developed to include species, such as birds, which were not measured, where their precise food requirements are known. The FSE results imply that, if introduced in a widespread and unmanaged way to UK agriculture in preference to their conventional counterparts, GMHT oilseed rape and beet could lead to further declines in farmland biodiversity (as indeed would any change in the management of these crops which reduce the weed burden in the crop). On the other hand, the widespread replacement of forage maize by its GMHT equivalent could be broadly beneficial for farmland wildlife.

Such generic conclusions do not consider other changes in crops or the agricultural landscape (between-crop differences in biodiversity being greater than those between GMHT and conventional crops) or allow for differences in take-up of the technology between different farm types (models of the impact of weed declines on skylark populations suggest that the outcome is sensitive to variation in the numbers of weeds already tolerated by different farmers<sup>20</sup>). They also discount the possibility of mitigating measures, either at the farm or regional scale, to enhance GMHT crops present a challange for the management of a countryside combining wildlife diversity and efficient food production

It is increasingly difficult to argue that remaining uncertainties carry sufficient risk to justify delaying the commercial release of GM crops which have received detailed regulatory scrutiny the value of nearby land for farmland biodiversity. Nonetheless the projected trends involved in introducing GMHT crops are clear for each crop and present an obvious challenge for the management of a countryside which combines wildlife diversity and efficient food production.

#### **CONCLUDING REMARKS**

Research published since 1999, and in particular the results of two very large and costly investigations on the Monarch butterfly in the USA and the impact of GM herbicide-tolerant crops on the UK, has done much to remove uncertainties about the large-scale cultivation of GM crops. There continues to be a need to assess each crop on a case-by-case basis while remaining vigilant to possible broader-scale environmental impacts. As with any new technology in agriculture or medicine, uncertainties remain. However, it is becoming increasingly difficult to argue on scientific grounds that these uncertainties carry sufficient risk to the environment to justify the continued delay to the commercial release of those crops that have received detailed regulatory scrutiny. These include the GMHT crops in the farm-scale trials. But of course science is merely one aspect of what is a complex political decision.

### References

- Gray, A. J. (1999), 'Genetically modified food crops: The environmental issues', J. Comm. Biotechnol., Vol. 6, pp. 154–159.
- Firbank, L. G. (2003), 'Introduction (one contribution of ten papers to a Theme Issue "The Farm Scale Evaluations of spring-sown genetically modified crops")', *Phil. Trans R. Soc. London*, Vol. 358, pp. 1777–1778.
- GM Science Review Panel (2003), 'GM Science Review. First Report', Department for Environment, Food & Rural Affairs, London.
- Beckie, H. J., Hall, L. M. and Warwick, S. I. (2001), 'Impact of herbicide-resistant crops as weeds in Canada', in 'Proceedings of Brighton Weed Conference', British Crop Protection Council, Farnham, pp. 135–142.
- Orson, J. (2002), 'Gene Stacking in Herbicide Tolerant Oilseed Rape: Lessons from the North American Experience', English Nature

Research Report No. 433, English Nature, Peterborough.

- Crawley, M. J., Brown, S. L., Hails, R. S. et al. (2001), 'Transgenic crops in natural habitats', *Nature*, Vol. 409, pp. 682–683.
- Linder, C. R. (1998), 'Potential persistence of transgenes: seed performance of transgenic canola and wild × canola hybrids', *Ecol. Applic.*, Vol. 8, pp. 1180–1195.
- Linder, C. R. and Schmitt, J. (1995), 'Potential persistence of escaped transgenes: Performance of transgenic oil-modified *Brassica* seeds and seedlings', *Ecol. Applic.*, Vol. 5, pp. 1050–1068.
- Gray, A. J. (2000), 'The transfer of traits to wild relatives', in 'Predicting Field Performance in Crop Protection', BCPC Symposium Proceedings No. 74, British Crop Protection Council, Farnham, pp. 165–174.
- Bullock, J. M. (1999), 'Using population matrix models to target GMO risk assessment', *Aspects Appl. Biol.*, Vol. 53, pp. 205–212.
- Warwick, S. I. et al. (2003), 'Hybridisation between Brassica napus L. and its wild relatives: B. rapa L, Raphanus raphinastrum L, Sinapis arvensis L and Erucastrum gallicum (Willd.) O.E. Schulz', Theor. Appl. Genet., Vol. 107, pp. 528–539.
- Jorgensen, R. B., Ammitzboll, H., Hansen, L. B. and Hanser, T. P. (2003), 'Gene introgression and consequences in *Brassica*', in 'Introgression from Genetically Modified Plants into Wild Relatives and its Consequences', Proceedings of an ESF Conference 21st–24th January, Amsterdam, in press.
- Desplanque, B., Hautekeete, N. and Van Dijk, H. (2002), 'Transgenic weed beets: possible, probable, avoidable', *J. Appl. Ecol.*, Vol. 39, pp. 561–571.
- Wilkinson, M. J., Elliott, L. J., Allainguillaume, J. *et al.* (2003), 'Hybridisation between *Brassica napus* and *B. rapa* on a national scale in the United Kingdom', *Science*, Vol. 302, pp. 457–459.
- Arnaud, J. F., Viard, F., Delescluse, M. and Cuguen, J. (2003), 'Evidence for gene flow via seed dispersal from crop to wild relatives in *Beta vulgaris* (Chenopodiaceae): consequences for the release of genetically modified crop species with weedy lineages', *Proc. R. Soc. Lond. B.*, Vol 70, pp. 1565–1571.
- Losey, J. E., Rayor, L. S. and Carter, M. E. (1999), 'Transgenic pollen harms Monarch larvae', *Nature*, Vol. 399, p. 214.
- Sears, M. K., Hellmich, R. L., Stanley-Horn, D. E. et al. (2001), 'Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment', *Proc. Natl Acad. Sci USA*, Vol. 98, pp. 11937–11942.

- Turner, R. G. (2004), 'The field-scale evaluation of herbicide-tolerant genetically modified crops conducted in the UK (1998– 2003)', *J. Comm. Biotechnol.*, Vol. 10, pp. 224–233.
- 19. Gray, A. J. (2004), 'Ecology and government

policies: The GM crops debate. The 12<sup>th</sup> BES Lecture', *J. Appl. Ecol.*, Vol. 41, pp. 1–10.

 Watkinson, A. R., Freckleton, R. P., Robinson, R. A. and Sutherland, W. J. (2000), 'Predicting biodiversity responses to GMherbicide-tolerant crops', *Science*, Vol. 289, pp. 1554–1556.