

## Gene flow from virus-resistant transgenic crops to wild relatives or to infecting viruses

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### ABSTRACT

The most important potential ecological risks associated with virus-resistant transgenic plants center on questions of gene flow. Gene flow from the transgenic plant to wild relatives could potentially confer a selective advantage on the wild plants, and perhaps increase their weediness. Gene flow from the transgene to an infecting virus by recombination could lead to new viral genomes. These questions need to be examined carefully, so that the potential risks can be correctly situated in a global risk/benefit analysis, which will take into account numerous other elements.

### INTRODUCTION

Gene flow from transgenic crops to wild relatives has been widely discussed in recent years. Attention has been primarily focused on certain economically important crops that have been transformed with herbicide resistance transgenes. For example, Mikkelsen *et al.*, (1996) have shown that a glufosinate tolerance transgene could be transmitted under field conditions from genetically engineered oilseed rape (*Brassica napus*) to its weedy relative, *Brassica rapa* (*B. campestris*). This raises the concern that dispersal of oilseed rape pollen over long distances (Timmons *et al.*, 1996) could lead to efficient dispersal of transgenes to wild species and by conferring herbicide resistance on the wild plants, limit the use of the herbicides involved. It has been proposed that biological containment of transgenes within transgenic crops can be achieved for some species through genetic engineering of the chloroplast genome (Daniell *et al.*, 1998). Unfortunately, chloroplasts, which are usually transmitted maternally, can also be inherited through pollen at a low frequency in many species, and therefore the use of chloroplast-transgenic plants cannot entirely prevent transgene flow (Stewart & Prakash, 1998). It is also true that gene flow will occur naturally in both directions, and that the result of pollination of rapeseed by *B. rapa* pollen will be exactly the same as that of the inverse cross. In any case, until a truly effective and universal system for genetic isolation of transgenic plants is developed, it remains pertinent to consider potential risks associated with gene flow between transgenic plants and sexually compatible wild relatives, but only for the few crop species where this is possible.

The concept of pathogen-derived resistance (Sanford & Johnson, 1985) applied to plant viruses paved the way to the development of virus-resistant transgenic (VRT) plants expressing various viral sequences, of which the first were reported in 1986 (for a review, see Beachy, 1997). The most widely used and best characterized viral transgenes are ones encoding a viral coat protein (CP). Nevertheless, many other viral genes, including ones encoding defective movement proteins, polymerases, proteases or helper components, as well as untranslatable viral sequences, have also provided high levels of resistance to viral infections when expressed in transgenic plants (Lomonosoff, 1995). In a relatively rapid move from the laboratory to the field, several VRT crop varieties have already been field tested and authorized for unrestricted commercial use in China and in the USA, and several more are to be released in the USA in the near future (White, 1999).

In preparation for deliberate small-scale and then unrestricted commercial release of VRT plants, several questions of potential risk have been raised (for a complete current overview, see Tepfer & Balázs, 1997). Ecological risks associated with VRT plants include synergism

with or heteroencapsidation of an infecting virus, which could lead to increased symptom severity or to spread of non-transmissible viruses, respectively. These risks can be considered to have potential effects that are simply phenotypic in nature, since they would remain restricted to the vicinity of the VRT crops and would disappear with no persistent effects on the environment if the VRT plants involved ceased being used. They will therefore not be further discussed here. VRT plants could also be a source of permanent genotypic modifications: (i) transmission of a resistance gene from transgenic crops to wild relatives could modify their natural competitiveness, or (ii) gene flow from transgenic plants to an infecting virus could lead to the emergence of recombinant viruses with modified biological properties, such as host specificity or symptomatology. Because of their potential for long-term, irreversible effects, these two potential genotypic risks will be discussed in more detail.

### GENE FLOW FROM TRANSGENIC VIRUS-RESISTANT CROPS TO WILD RELATIVES

Considering the potential impacts of gene flow is of course pertinent for the rare VRT plant species that can be expected to be cultivated in proximity to compatible wild relatives. The situation with VRT plants is quite different from that of herbicide-resistant ones, where selection pressure, i.e. herbicide use, is entirely determined by human activity. The selection pressure exerted by viruses, and by other biological or environmental stresses, is entirely beyond human control and has rarely been studied. The critical question is, if gene flow from VRT plants to wild relatives does occur, to what extent will this confer a selective advantage on the VRT wild plants?

Questions relative to gene flow from VRT crops to wild plants are currently being studied with two experimental systems, involving either sugar beet or squash. There is clear evidence for natural gene flow between cultivated sugar beets (*Beta vulgaris* ssp *vulgaris* provar. *altissima* Döll) and wild beets (*B. vulgaris* ssp *maritima* Arcang.), which are perfectly compatible (Boudry *et al.*, 1993), and also between the cultivated squash, *Cucurbita pepo* var. *ovifera*, and the wild squash, *C. pepo* var. *texana* (Fuchs & Gonsalves, 1997). As would be expected, Bartsch *et al.* (1996) have shown that transgenic sugar beet resistant to beet necrotic yellow vein virus (BNYVV) has an ecological advantage over untransformed controls when grown under field conditions, particularly when there was heavy virus infestation. The effects of gene flow in the squash system have been studied in more detail. Fuchs & Gonsalves (1997) have shown that transgene flow does occur between VRT cultivated squash expressing the coat protein genes of cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV2) and its wild relative under conditions of low disease pressure. Both F1 hybrid squash plants and plants of the BC1 generation exhibited greater fitness than wild-type plants when tested under conditions of high disease pressure, but not under ones of low disease pressure. They also found that certain BC1 individuals were phenotypically indistinguishable from wild squash, and thus could be predicted to survive under non-cultivated conditions.

The crucial (and still unresolved) question is whether a virus-resistant wild squash or beet can become a significantly greater threat as an invasive weed. Unfortunately, very few studies on the impact of viral infections on wild plants have been undertaken. Among the few examples, one can cite the experiments showing that under controlled glasshouse conditions CMV reduces the competitive capacity of both purslane (*Portulaca oleracea*) and chickweed (*Stellaria media*) through an inhibition of growth and/or a reduction of reproductive potential (Friess & Maillet, 1996; 1997). Even less is known about the effects on fitness of wild plants when grown under natural conditions. The study of Kelley (1993) on *Anthoxanthum odoratum* infected with barley yellow dwarf virus (BYDV) is perhaps the unique example. He showed that even though BYDV infection did not induce visible symptoms on *A. odoratum*, it did have a clear negative effect on fitness, as reflected in fecundity.

These results show that viral infection can decrease plant fitness, and tend to support the hypothesis that a virus-resistant phenotype, whether natural or acquired through genetic engineering, could confer a selective advantage. However, the situation under non-cultivated conditions may be more complex. For instance, even though *C. pepo* var. *texana* exhibits pronounced symptoms when infected in the glasshouse or experimental field, the prevalence of the target viruses is apparently extremely low in native populations (Fuchs & Gonsalves, 1997). Similarly, in a modest sampling of wild beet populations in Italy, no BNYVV was detected (Bartsch, 1997). If more detailed studies show that virus is generally absent from these species, for reasons that remain to be determined (other forms of resistance, absence of vectors, lack of source plants, etc.), one would predict that in fact introgression of a virus resistance gene would be expected to confer no selective advantage, and would have no effect on the weediness of the wild plants.

### GENE FLOW FROM TRANSGENIC PLANTS TO AN INFECTING VIRUS AS A SOURCE OF VARIABILITY IN VIRAL GENOMES

Analysis of viral genomic sequence data often shows genetic features that can be best attributed to RNA recombination events between viruses (Revers *et al.*, 1996) or between viruses and plant RNAs (Mayo and Jolly, 1991; Masuta *et al.*, 1992), indicating that RNA recombination is a normal feature of virus evolution. Thus, the use of viral sequences in transgenic plants immediately raised the question of whether recombination could occur between viral transgene sequences and the genome of an infecting virus, and whether this could have an impact on virus evolution.

In 1994, Greene & Allison showed that recombination between transgene-derived mRNA encoding the 3' two-thirds of the viral coat protein (CP) of cowpea chlorotic mosaic virus (CCMV) and an isolate of CCMV that had been crippled by the deletion of the 3' one-third of its CP gene, could give rise to viable viral progeny *via* RNA recombination. The viral genomes recovered were apparently created by imprecise homologous recombination, and also had minor mutations surrounding the site of crossover. When tested on a range of host plants, several of the recombinant strains caused novel symptoms (Allison *et al.*, 1997), but none was more fit than the parental strain when co-inoculated with wild-type virus (Allison *et al.*, 1999). These results demonstrate that recombination can occur between transgene-derived viral sequences and the genome of an infecting virus. However, these recombinant strains were obtained under conditions radically different from those that would occur in the field, since conditions of high selection pressure was exerted in favor of recombination events that restore viability. Working under conditions of moderate selection pressure, i.e. with an intact cauliflower mosaic virus (CaMV) genome as the infecting strain, Wintermantel *et al.* (1996) showed that inoculation of transgenic *N. bigelovii* plants expressing D4-CaMV ORF IV with W260-CaMV, which is systemic in solanaceous hosts, generated recombinant viruses that had a distinct competitive advantage in *N. bigelovii* when compared to the parental W260 strain. These results demonstrate that gene flow from transgenic plants to infecting viruses can occur, although at very low levels and under particular conditions.

In order to estimate the potential risks of VRT plants regarding virus recombination, it has often been proposed to apply a classic formula of risk assessment, in which risk = hazard X frequency (Hull, 1994). In the context of recombination in VRT plants, application of this formula would require evaluation of the pathogenicity of recombinant viruses (hazard), and also determination of the frequency of their occurrence.

Creating recombinant viruses *in vitro* by exchanging RNA segments of different strains or of related viruses and evaluating their biological properties is currently the best way to study the potential hazard associated with recombinant viruses. Certain artificial recombinant cucumoviruses show striking changes in symptomatology (Ding *et al.*, 1996;

Salánki *et al.*, 1997; Carrère *et al.*, 1999), but their fitness has not been compared to that of the parental viruses. There is however one report of a recombinant cucumovirus that shows an increased fitness when compared to its parental strains (Fernández-Cuartero *et al.*, 1994). This particular recombinant appeared spontaneously after several years of maintenance in the greenhouse of a pseudo-recombinant strain composed of CMV RNAs 1 and 2 and tomato aspermy virus (TAV) RNA3, which is rather far from the conditions encountered during field cultivation of VRT plants. None of these reports can give a clear picture of the impact of recombinants between the transcripts of a viral transgene expressed in a VRT plant and a viral genome, in particular because artificial recombinants are created by exchanging homologous restriction fragments between cloned viruses. Creating random recombinants between the transcripts of a viral transgene and the genome of a potential infecting virus, then comparing the properties (aggressivity, host range, fitness) of recombinants to those of the parental viruses would be the better way to estimate the hazards of potential recombinants between VRT plants and viruses. To this end, we are currently exploring techniques for creating a random library of recombinant cucumoviral genomes.

The frequency of recombination is also difficult to establish, particularly since strictly speaking it can only be determined under conditions of the lowest possible selection pressure. Otherwise, counter-selection of non-viable recombinants leads to a systematic underestimation of frequency. In addition, since recombination between viral genomes is a normal phenomenon, the frequency of its occurrence in virus-infected VRT plants should be compared to that in doubly-infected non-transgenic plants. To this end, molecular strategies have been developed to detect recombinant cucumoviruses under little or no selection pressure (Aaziz & Tepfer, 1999). Using these techniques, recombinants between genomic RNAs of two cucumoviruses, CMV and TAV, have recently been observed in coinfecting plants in the absence of selection pressure (Aaziz & Tepfer, submitted). The objective of current work is to study RNA recombination in the corresponding CP-CMV plants.

## CONCLUSIONS

Upon re-examination of the initially proposed risk assessment formula (risk = hazard X frequency), it becomes apparent that in fact this may not be the appropriate model for the types of risk under consideration here. Compared for instance to most industrial risks, or to the risk of damage to an automobile in an accident, the potential genotypic risks associated with VRT plants differ by giving rise to changes that could be irreversible. Thus, we would propose that the only important questions are: can the suspected event occur, and if so, what are the consequences? The frequency of the event will in fact only affect the rapidity of appearance of its effects. In the types of gene flow under consideration here, this would mean that once it has been determined that the frequency is greater than zero, the important points would be to determine if the transgene can confer enhanced fitness, but also whether there is indeed selection pressure in nature that would give an advantage to the plant or virus with the novel genotype.

The potential risks associated with VRT plants are all due to natural phenomena. Gene flow between certain crop species and wild relatives has always occurred, as has recombination between viral genomes. It is thus of great importance to select the most appropriate baseline against which to compare the potential risks. For instance, not knowing the baseline impact of virus infection on a wild species, we cannot predict the possible impact of introgression of a resistance gene from a related VRT crop plant. Here, as in the area of recombination between viral genomes, more thorough understanding of the basics is necessary.

In closing, we would like to consider how to make the appropriate decision if future studies lead to the conclusion that VRT plants do present some level of ecological risks due to gene flow. Since zero risk does not exist in this domain, as in any other, we need to have the

means to determine if the risk in question is acceptable or not. This broader evaluation has been made more difficult by the widely held misconception that the principle of precaution implies acceptance of only zero risk. The decision on deployment of a given VRT plant clearly involves a risk/benefit analysis, which will not only include evaluation of risk as determined in as scientifically rigorous a fashion as possible, but will also include consideration of the alternatives to not deploying the VRT plants. In a region of the world that suffers more from agricultural overproduction than from shortage, the need for more and better virus resistance is not always obvious. Nonetheless, even in Europe there are serious viral diseases against which natural resistance genes are not available. In these cases, deployment of VRT plants would make it unnecessary to use the pesticides that are currently used to eliminate the organisms that are vectors of viral disease (insects, fungi and nematodes), and would also lead to enhanced quality of the agricultural products. Viral diseases also have an important impact on the yield of certain major crops, such as rice. It should also be remembered that in a perhaps not so distant future, the global demographic pressure may make it necessary to use all available means for increasing agricultural productivity, and this may well include VRT plants.

## ACKNOWLEDGEMENTS

P-Y Teycheney is supported by a fellowship from the Association pour la Recherche sur les Nicotianées.

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## Interactions between insect tolerant genetically modified plants and natural enemies

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### ABSTRACT

Insect natural enemies play an important role in the regulation of many pest species. The effects of genetically modified (GM) crops on natural enemies and other non-target species need to be evaluated to avoid disruption of natural biological control and ecosystem dynamics. This paper reviews progress with studying effects of GM plants on tritrophic systems involving parasitoids and predators. Experiments at Rothamsted using mixtures of GM and wildtype oilseed rape plants in large cages have shown no differences in parasitism rates of the aphid *Myzus persicae* by the parasitoid *Diaeretiella rapae* on GM and untransformed plants. Ongoing experiments are investigating the effect of GM oilseed rape on the parasitisation and host searching behaviour of *Cotesia plutellae*, a larval parasitoid of the diamondback moth (*Plutella xylostella*).

### INTRODUCTION

All currently commercialized insect tolerant genetically modified (GM) crops (cotton, maize and potato) express  $\delta$ -endotoxin genes derived from the bacterium *Bacillus thuringiensis* (*Bt*). In addition to *Bt* genes, an increasing number of other potential insect resistance genes are being transferred into crop cultivars, such as genes coding for cholesterol oxidase, lectins (e.g. the snowdrop lectin, GNA) and inhibitors of digestive enzymes such as proteinase inhibitors (e.g. the cowpea trypsin inhibitor, CpTI) and amylase inhibitors. Constitutive promoters have been used to drive gene expression in the majority of currently existing insect tolerant GM plants leading to gene expression in most tissues of the plant (Schuler *et al.*, 1998). Most herbivorous insects feeding on GM plants are therefore likely to ingest these heterologous proteins.

Agricultural crops support not only pest insects but also beneficial insects which feed on these herbivores and which play an important role in the regulation of pest populations (van Driesche & Bellows Jr, 1996). Current pest control measures usually involve the use of broad spectrum synthetic insecticides which act on contact and are often highly toxic to these beneficial insects. Insect tolerant GM plants are expected to be more benign for beneficial insects, but they represent a new technology and interactions between these plants and the third trophic level have to be tested to avoid any unnecessary disruption of natural biological control or ecosystem dynamics.

The impact of insect tolerant plants on the population dynamics of natural enemies will depend on many factors such as the level of tolerance of the plant to the pest species, the scale on which the GM crop is grown and the susceptibility, mobility and host range of the natural enemy. The most obvious way highly tolerant crops can affect natural enemies is by

severely depleting their supply of prey or hosts. Such effects will be most profound for specialist natural enemies that feed exclusively on insects the GM plant is designed to kill, a fact that applies to all pest control measures and which is by no means unique to GM plants. When highly tolerant plants are grown on a larger scale the abundance of some natural enemies may therefore decline due to prey depletion, but their persistence is not necessarily threatened if other nearby crops or weeds support acceptable prey species, or if their host range includes species other than the target pests. However, not all herbivorous insects feeding on GM plants will receive lethal doses, either because (a) a heterologous protein only provides a low level of tolerance, (b) the expression level of the heterologous protein is low or variable, or (c) a herbivore is not very susceptible to the protein (either inherently as a species or because it has developed resistance). Thus, in addition to the effects of prey depletion, the potentially more subtle effects of sublethal doses on tritrophic interactions involving predators and parasitoids need to be investigated especially if herbivores can accumulate the toxin without deleterious effects.

## PARASITOIDS

Parasitoids are mainly restricted to the insect orders Hymenoptera and Diptera. They complete their larval development on or in a single host insect which is killed in the process (Godfray, 1994). Compared with predators, parasitoids are relatively host-specific; some species even depend solely on a particular stage of a single host species.

Since parasitoid larvae depend on one host individual, any pest control measure, including GM plants, that kills the host before the parasitoid has completed its development will result in the death of the parasitoid. *Bt* oilseed rape, for example, can cause 100% mortality of *Bt*-susceptible diamondback moth (*Plutella xylostella*) larvae (Stewart Jr, 1996) and, as a direct consequence, larval parasitoids of this pest, such as the braconid *Cotesia plutellae*, are unable to complete their development. In contrast, preliminary results suggest that survival of *C. plutellae* in *Bt*-resistant *P. xylostella* was not affected by the host having fed on *Bt* oilseed rape (Figure 1).

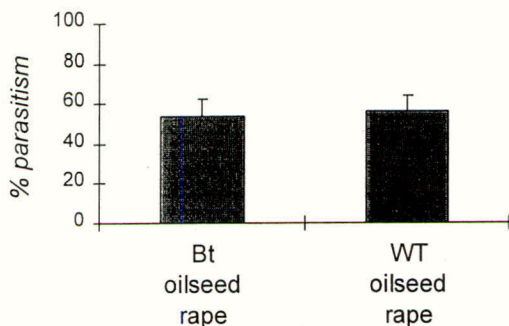


Figure 1. Parasitism of *Bt*-resistant diamondback moth larvae by *Cotesia plutellae* on *Bt* oilseed rape compared to wildtype (WT) oilseed rape of the same cultivar



It is known from studies using conventionally-bred tolerant plants and also from work with microbial *Bt* insecticides, that sublethal doses have the potential for synergistic, antagonistic or neutral interactions with parasitoids. For example, less vigorous host defence caused by sublethal poisoning potentially increases oviposition opportunities for parasitoids. There is also the possibility that sublethal toxin levels improve levels of parasitism by impairing the host's immune response to parasitism. In contrast, sublethal doses could lead to a reduced host quality, and thereby to a reduced fitness of parasitoids developing on such hosts. Parasitoid larvae might also be exposed directly to the heterologous protein when consuming host tissue. Further detailed studies are required to unravel these complex interactions.

Parasitoids of non-target pest species have received little attention so far but GM plants have the potential to affect these as well. Aphid populations, for example, are not controlled by *Bt* plants. Parasitoids are important natural antagonists of aphids and any negative side effects on these parasitoids could lead to increased problems with aphid pests. We are, therefore, investigating the effect of mixtures of *Bt* oilseed rape and wildtype oilseed rape on the aphid parasitoid *Diaeretiella rapae* on a population scale in large laboratory cages. Our results so far indicate that aphid parasitoids are as efficient in controlling aphid populations on *Bt* oilseed rape as on wildtype oilseed rape (Figure 2).

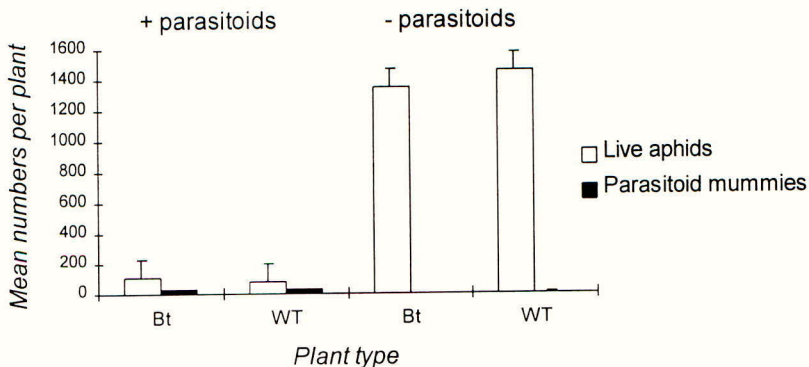


Figure 2. Population size of *Myzus persicae* and parasitism by *Diaeretiella rapae* reared for one parasitoid generation on mixtures of *Bt* oilseed rape and wildtype (WT) oilseed rape in large cages (1.7m x 1.2m x 1.0m) in the laboratory. Populations were initiated by placing fifteen apterous adult aphids on each plant (four plants of each type per cage) and, two days later, releasing seventy-two *D. rapae* females into each cage ('+ parasitoids'). No parasitoids were added to the control cages ('- parasitoids').

When locating hosts, female parasitoids use volatile stimuli released from plants, especially when the plant is damaged by herbivores (Vet & Dicke, 1992). The quantity and/or composition of the emitted plant volatiles can vary between plant species and between genotypes of the same plant species (Dicke & Vet, 1998). It has been demonstrated that several parasitoid species use these volatile cues to discriminate in-flight between different plant species or even individual cultivars (Vet & Dicke, 1992). The genetic modification of plants could conceivably affect the volatile profile released by the plant, and subsequently influence the attractiveness for beneficial insects such as parasitoids. To investigate whether

parasitoids differ in their response to GM plants, we have begun to test the response of the parasitoid *C. plutellae* to uninfested oilseed rape leaves in a windtunnel. *C. plutellae* adults were attracted to both *Bt* oilseed rape and oilseed rape transformed with the proteinase inhibitor OC-I. The parasitoids did not distinguish between control and these GM plants when offered in a choice situation (Potting, unpublished data).

A number of field experiments have investigated the effect of *Bt* plants on parasitoids on a larger scale. Orr and Landis (1997) planted microplots of untransformed maize within large plots of *Bt* maize and infested them artificially with eggs of the European cornborer (*Ostrinia nubilalis*). This method was used since *O. nubilalis* larvae do not survive on *Bt* maize. Levels of parasitism of *O. nubilalis* larvae collected from these microplots did not differ between *Bt* and untransformed plots. Mascarenhas and Luttrell (1997) studied the combined effect of sublethal exposure to *Bt* cotton and natural enemies on the survival of bollworm larvae (*Helicoverpa zea*). Sublethal exposure was achieved by rearing larvae for one to four days on *Bt* cotton in the laboratory before transferring the surviving larvae to untransformed cotton in the field. There was no difference in survival of *H. zea* larvae from GM and untransformed plants when natural enemies were excluded. However, when natural enemies were present, larvae exposed to sublethal doses of *Bt* cotton survived at lower rates than larvae reared entirely on untransformed cotton. Field trials with low-expressing *Bt* tobacco lines indicated a synergistic interaction between the transgenic plants and the ichneumonid wasp *Camponotus sonorensis* in controlling tobacco budworm larvae (*Heliothis virescens*), probably partly due to a slower larval development of the host (Johnson, 1997; Johnson *et al.*, 1997).

## PREDATORY INSECTS

Predatory insects can be found in most insect orders including the Coleoptera (e.g. Carabidae, Coccinellidae), Heteroptera (e.g. Anthocoridae, Nabidae), Neuroptera (e.g. Chrysopidae), Hymenoptera (e.g. Vespidae, Formicidae) and Diptera (e.g. Syrphidae) (van Driesche & Bellows Jr, 1996). Predatory species of importance in a crop environment need to consume large numbers of pest insects to complete their life cycle. Both the larval and the adult stages are mobile and in some species both are predatory while in other species only the larvae are carnivorous while the adults feed on flowers. Most predators feed on a range of different insect species.

A number of laboratory and field studies have been conducted to investigate interactions between GM plants and predatory insects. Dogan *et al.* (1996) studied the effect of *Bt* potatoes on the ladybird *Hippodamia convergens* in the laboratory. No significant effects on survival, consumption of aphids (*Myzus persicae*), development or reproduction in ladybirds were observed. Pilcher *et al.* (1997) fed pollen of *Bt* maize to the ladybird *Coleomegilla maculata*, the anthocorid bug *Orius insidiosus* and the lacewing *Crysopa carnea*, and found no acute detrimental effects on preimaginal development and survival. In a laboratory study by Sims (1995) a concentration of 20 µg ml<sup>-1</sup> activated *Bt* toxin applied to prey or in honey solution, did not affect *C. carnea* or *H. convergens*, respectively. Hilbeck *et al.* (1998), however, reported that feeding prey that had been reared on *Bt* maize to *C. carnea* in the laboratory increased larval mortality of the predator from 37% (control) to 62%. In this study, two species of prey were tested, the European cornborer (the susceptible target species) and the Egyptian leafworm, *Spodoptera littoralis* (a less susceptible species).

Microbial *Bt* insecticides are generally perceived as safe for beneficial insects and are an essential component of many integrated pest management programmes. Some studies have, however, demonstrated that microbial *Bt* insecticides can be detrimental to certain natural enemies (reviewed by Croft and Flexner, 1990).

Birch *et al.* (in press) investigated potential side effects of potatoes expressing the lectin GNA on the two spotted ladybird (*Adalia bipunctata*). GNA potatoes provide low levels of resistance to a range of pest insects including aphids. Peach potato aphids (*Myzus persicae*) were reared on GNA potatoes and then fed to ladybirds. Compared to control plants the GNA potatoes resulted in a 38% reduced fecundity of ladybird females; these females only lived half as long as females on control plants and the viability of their eggs was reduced three to four-fold. It remains to be clarified whether these negative effects were caused by a direct effect of the GNA on the ladybirds or if they were due to a reduction in prey quality.

In the field no negative side effects on predatory insects have been reported and in some cases significantly greater numbers of predators were found on *Bt* plants compared to untransformed plants. Hoffmann *et al.* (1992) monitored predatory bugs of the family Nabidae on *Bt* and CpTI tobacco in the field. Numbers of Nabidae were greater on both types of GM plants compared to the control plots but the differences were not significant. Similarly, no detrimental effects were observed on the abundance of coccinellid, anthocorid and chrysopid predators of *O. nubilalis* on transgenic *Bt* maize in a two-year field experiment (Pilcher *et al.*, 1997). In another large scale field study, Orr and Landis (1997) found no negative effects of *Bt* maize on egg predation or predator densities, and on some sampling occasions numbers of lacewings and ladybirds were significantly higher on *Bt* maize compared to untransformed maize. The negative side effects on lacewings described in the laboratory (Hilbeck *et al.*, 1998) have so far not been confirmed in the field. This might be due to the fact that in maize crops *C. carnea* tends to feed mainly on aphids while the stem-boring *O. nubilalis* larva is hardly exposed to predation by lacewings. Monitoring of *Bt* cotton has so far also failed to show any significant effects on predators, including *C. carnea* (Flint *et al.*, 1995; Wilson *et al.*, 1992).

## CONCLUSIONS

So far, reports of negative side effects of GM plants on natural enemies have been confined to small scale tests in the laboratory but field studies have not shown any negative effects on beneficial insects. Future research needs to include work on more tritrophic systems, clarification of the mechanisms behind observed detrimental effects, further studies to relate data from laboratory bioassays to the population and field level and, finally, comparison of effects of GM plants with those of conventional pest control measures. Vigorous and standardised risk assessment methodologies have to be applied. Post-release monitoring of natural enemies in GM crops will also be essential.

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