Review

Will transgenic plants adversely affect the environment?

VASSILI V VELKOV [1,*], ALEXANDER B MEDVINSKY [2], MIKHAIL S SOKOLOV [3] and ANATOLY I MARCHENKO [3]

[1]*Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russian Federation*

[2]*Institute of Theoretical & Experimental Biophysicis, Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russian Federation*

[3]*Research Center for Toxicology and Hygienic Regulation of Biopreparations, Ministry of Health of the Russian Federation, Serpukhov, Moscow Region, 142253, Russian Federation*

**Corresponding author (Fax, (0967) 330-528; Email, vvvelkov@rambler.ru)*

Transgenic insecticidal plants based on *Bacillus thuringiensis* (Bt) endotoxins, on proteinase inhibitors and on lectins, and transgenic herbicide tolerant plants are widely used in modern agriculture. The results of the studies on likelihood and non-likelihood of adverse effects of transgenic plants on the environment including: (i) effects on nontarget species; (ii) invasiveness; (iii) potential for transgenes to 'escape' into the environment by horizontal gene transfer; and (iv) adverse effects on soil biota are reviewed. In general, it seems that large-scale implementation of transgenic insecticidal and herbicide tolerant plants do not display considerable negative effects on the environments and, moreover, at least some transgenic plants can improve the corresponding environments and human health because their production considerably reduces the load of chemical insecticides and herbicides.

[Velkov V V, Medvinsky A B, Sokolov M S and Marchenko A I 2005 Will transgenic plants adversely affect the environment?; *J. Biosci.* **30** 515–548]

1. Introduction

Transgenic plants (TPs) display considerable potential to benefit both developed and developing countries. Expressing insecticidal proteins, or proteins providing tolerance to herbicides or resistance to environmental stresses TPs are revolutionizing argriculture (Carpenter and Gianessi 2000; Shelton *et al* 2002; Wang *et al* 2003). The use of such crops with input traits for pest management – primarily insect and herbicide resistance has risen dramatically since their introduction in the mid-1990's. In recent years regulations have been developed to address the risks of releasing TPs into the natural environment. It is clear that future agricultural and, ultimately, also natural ecosystems will be challenged by the large-scale introduction of genetically modified organisms, containing entirely novel genes and gene products in new combinations at high frequencies all of which will have unknown impacts on their associated complex of non-target organisms, i.e. all organisms that are not targeted by the insecticidal protein produced by the TPs. In times of severe global decline of biodiversity, pro-active precaution is

Keywords. Biodiversity; environment; ecology; genetically modified plants; human health; introgression; transgenic plants

Abbreviations used: AFLP, Amplified fragment length polymorphism; AM, arbuscular mycorrhizal; BBPs, biotin-binding proteins; Bt, *Bacillus thuringiensis*; CEWc, chicken egg white cystatin; CFU, colony forming unit; CPB, Colorado potato beetle; CpTi, cowpea trypsin inhibitor; ECB, European corn borer; GFP, green fluorescent protein; GNA, *Galanthus nivalis* agglutinin; GPA, green peach aphid; HT, herbicide tolerant; MIT-2, mustard trypsin inhibitor-2; PCN, potato cyst hematodes; PCR, polymerase chain reaction; PIs, protease inhibitors; PLFA, phospholipid fatty acid analysis; rNPT-II, cloned neomycin phosphotransferase-II; SBTI, soybean trypsin inhibitor; SSCP, single-strand conformation polymorphism; TPs, transgenic plants.

necessary and careful consideration of the likely expected effects of TPs on biodiversity of plants and insects is mandatory. (Wolfenbarger and Phifer 2000; Velkov *et al* 2003a,b). To ensure safe crops to humans and the environment, a strong, but not stifling, regulatory system needs to be established and properly implemented. The major components of those systems are: (i) mandatory pre-market approval; (ii) established safety standards; (iii) transparency; (iv) public participation; (v) use of outside scientists for expert scientific advice; (vi) independent agency decisions; (vii) post-approval activities; and (viii) enforcement authority and resources. Although none of the existing systems adequately achieves all the necessary components of a strong regulatory system, those systems serve as models for deciding which regulatory procedures should be emulated and which should be avoided. A mandatory pre-market approval system that applies established safety standards in procedures that are transparent and allows for public participation with no pre-conceived notions or biases will best achieve both safe products and consumer trust. (reviewed in Marvier 2002; Giovannetti 2003; Velkov et al 2003a,b; Jaffe 2004).

In spite of considerable attempts of governmental regulators to adopt such procedures the debates in scientific and public communities, which addresses both safety and 'precautionary approach,' has raised questions about the impact of TPs on the biodiversity of traditional landraces, on the agricultural and natural ecosystems and on the environment in general (reviewed in Wisniewski *et al* 2002; Mendelsohn *et al* 2003; Conko 2003).

Governmental authorities responsible to say the final *yes* or *no* to large-scale implementation of TPs expect from scientists, responsible for the corresponding environmental risk assessments the answers formulated in the terms *yes* or *no* and without reserves. Such requirement is reasonable, because the majority of TPs are especially designed to have ecological (and then economical) advantageous over their non transgenic parents and relatives. Generally speaking, these TPs could be characterized as *resistant*: resistant to harmful insects (insecticidal TPs), to herbicides (herbicide tolerant, HT), to salt stress (salt tolerant), to drought (drought tolerant). But what are the chances that these TPs will bring also unintended disadvantageous? Will such TPs displace their relatives not armed with corresponding transgens? Or will, for example, insecticidal TPs kill non target (beneficial) organisms and disrupt essential food chains? Will transgenes escape form TPs via transgenic pollen dissemination or by microbes capturing transgenes? Neither any sophisticated speculation nor common sense reasoning can answer these questions, but only non-ambiguous results of experimental studies collected, analysed and generalized. Let's see how it is in fact.

2. Will insecticidal TPs kill non target organisms?

Although insecticidal TPs usually are constructed to kill only a narrow spectrum of lepidopteran species, they could directly or indirectly damage non-target species also. A lot of testing methods have been developed for assessing the impact of TPs on non-target organisms. These tests begin in contained, indoor conditions, and often use transgene products rather than TPs and progress to more realistic conditions, involving microcosms, field trials, modelling, and finally farm-scale evaluations or commercial crop monitoring. Non-target organisms are selected on a range of criteria (e.g. abundance in the field, ease of handling in the laboratory, taxonomic certainty, value to the agricultural ecosystem, endangered status), and those for which toxicity is demonstrated are then subjected to more detailed investigation (Cowgill and Atkinson 2003; Dutton *et al* 2003a,b; Schmitz *et al* 2003). Four Bt delta-endotoxin genes (*cry1Ab*, *cry1Ac*, *cry2Ab*, and *cry9C*) are currently used commercially in corn and cotton to protect against lepidopteran pest attack (Shelton *et al* 2002). Other transgenes coding proteins with insecticidal activities widely used for transgenic plants developing are: (i) protease inhibitors (PIs) (Cowgill and Atkinson 2003); (ii) lectins, whose ranges of insecticidal activity are generally broader than those of Bt toxins (Griffiths *et al* 2000); (iii) biotin-binding proteins (BBPs), which have a broad spectrum of insect toxicity (Kramer *et al* 2000; Burgess *et al* 2002); (iv) toxins from bacterial symbionts of entomopathogenic nematodes (Kramer *et al* 2001); (v) chitinases (Wang *et al* 1996); and (vi) enhancin from insects (Cao *et al* 2002). Usually such TPs are designated as protected by the corresponding transgenes.

There is widespread concern that insecticidal TPs will cause mortality in non-target insects. Is this kind of risk real?

(i) *Yes*

Bt protected TPs: The first evidence that this may be the case was observed when Bt corn (Cry1Ab) pollen naturally deposited on *Asclepias syriaca*; common milkweed, in a corn field caused significant mortality of *Danaus plexippus* L. (Lepidoptera: Danaidae) larvae. It was shown that larvae feeding for 48 h on *A. syriaca* plants naturally dusted with pollen from Bt corn suffered significantly higher rates of mortality at 48 h ($20 \pm 3\%$) compared to larvae feeding on leaves with no pollen ($3 \pm 3\%$), or feeding on leaves with non-Bt pollen (0%). Mortality at 120 h of *D. plexippus* larvae exposed to 135 pollen grains/cm^2 of transgenic pollen for 48 h ranged from 37 to 70%. Based on the quantification of the wind dispersal of this pollen beyond the edges of agricultural fields, it was predicted that the effects of transgenic pollen on *D. plexippus* may be observed at least 10 m from transgenic field

borders. However, the highest larval mortality occurred on *A. syriaca* plants in corn fields or within 3 m of the edge of a transgenic corn field (Jesse and Obrycki 2000). According to the next field study of the impact of exposure to pollen from a Bt corn (Cry1Ab, event 176) on two species of Lepidoptera, black swallowtails and monarch butterflies (*D. plexippus*), it was documented that nearly half of the 600 monarch larvae died within the first 24 h: this and subsequent mortality was not associated with proximity to Bt corn and may have been due in part to predation. Survivorship of black swallowtails was much higher than that of the monarchs and was also independent of proximity to the transgenic corn. However, despite five rainfall events that removed much of the pollen from the leaves of their host plants during the experiment, a significant reduction in growth rates of black swallowtail larvae was observed that was likely caused by pollen exposure. Authors claimed that these results suggested that Bt corn can have adverse sublethal effects on black swallowtails in the field (Zangerl *et al* 2001).

TPs protected by antimicrobial genes: A similar results was obtained when the biology and behaviour of pear psylla, *Cacopsylla pyricola* Foerster, on a transgenic 'Bartlett' pear, *Pyrus communis* L., containing a synthetic antimicrobial gene, D5C1, was compared with that of a non-transgenic parental plant to determine whether there were any nontarget effects. The gene-construct also contained the marker gene *nptII* (aminoglycoside 3′-phosphotransferase II) that encodes for antibiotic resistance to identify transformed plants. The purpose of the genetical modification was to enhance pear resistance to the bacterial disease fireblight caused by *Erwinia amylovora* (Burr.). The biology and behaviour of pear psylla on TPs were compared with a nontransgenic parental pear in short-term (less than or equal to 7 days) and long-term (32 days) studies. Short-term studies indicated pear psylla adults preferred to settle and oviposit, and nymphs fed more and developed slightly faster, on transgenic pear compared with nontransgenic pear. In contrast, a long-term study on psylla colony development showed considerably fewer eggs, nymphs, and adults were produced on transgenic pear. Although adults reared on transgenic pear did not have weight affected, females produced fewer eggs and nymphal hatch was significantly reduced on the transgenic pear. In general, the results suggested that pear-psylla biology and behaviour are initially enhanced on this TP. However, chronic exposure of psylla populations to the TPs that express the *nptII* marker and lytic peptide genes had detrimental effects on pear psylla reproductive biology. Overall, this study demonstrated that TPs used to control one particular organism can have unintentional yet beneficial effects against other nontarget pest organisms in agricultural crops (Puterka *et al* 2002).

(ii) *No*

Bt protected TPs: Purified preparations of a *B. thuringiensis* var. kurstaki CryIA(c) protein equivalent to the insecticidal protein produced by transgenic cotton were tested against 14 species of insects – (i) Coleoptera: *Anthonomus grandis* Boheman, *Diabrotica undecimpunctata* howardi Barber, *Leptinotarsa decemlineata* (Say), *Hippodamia convergens* Guerin-Meneville; (ii) Diptera: *Aedes aegypti* (L.); (iii) Homoptera: *Myzus persicae* (Sulzer); (iv) Hymenoptera: *Apis mellifera* L., *Nasonia vitripennis* (Walker); (v) Lepidoptera: *Ostrinia nubilalis* (Hubner); *Manduca sexta* (L.), *Helicoverpa tea* (Boddie), *Heliothis virescens* (Fabr.); (vi) Neuroptera: Chrysopa carnea Stephens; and (vii) Orthoptera: *Blattella germanica* (L.). Ten species were tested using diet-incorporation feeding bioassays exposing insects to a high dose concentration of 100 µg/ml of the 'full-length' CryIA(c) protein. Four beneficial insect species (*A. mellifera*, *C. carnea*, *H. convergens*, and *N. vitripennis*) were tested at ca. 20 µg/ml concentration in diet. This concentration was > 100 times the concentration of CryIA(c) protein found in the field as present in pollen and nectar of transgenic cotton. Of the 14 insect species tested, only four species of Lepidoptera had > 50% mortality.The mortality of non-lepidoptera species exposed to the proteins did not differ significantly from control mortality. It was concluded, that the CryIA(c) protein expressed in transgenic cotton has biological activity specific for Lepidoptera and that risks to beneficial non-lepidoptera insect species are negligible (Sims 1995). Among the lepidopterans at high potential risk from Bt protected plants is the black swallowtail butterfly *Papilio polyxenes*. A field study was performed to assess whether mortality of early instar black swallowtails was associated either with proximity to a field of Bt corn or by levels of Bt pollen deposition on host plants. Potted host plants were infested with first instar black swallowtails and placed at intervals from the edge of a field of Bt corn. The pollen from these plants contained Cry1Ab endotoxin ($2{\cdot}125 \pm 0{\cdot}289$ ng/g). Although many of the larvae died during the 7 days that the experiments were run, there was no relationship between mortality and proximity to the field or pollen deposition on host plants. Moreover, pollen from these same plants failed to cause mortality in the laboratory at the highest pollen dose tested (10,000 grains/cm^2), a level that far exceeded the highest pollen density observed in the field (200 grains/cm^2). It was concluded that Bt pollen of the variety tested is unlikely to affect wild populations of black swallowtails (Wraight *et al* 2000).

In contrast to the initial investigations of the negative impact of Bt corn (Cry1Ab) on *D. plexippus* (which provoked strong negative public response to transgenic biotechnology) in the next experiments the 'Monarch case'

was revised and the verdict was returned. The effect of Bt corn proximity and Bt corn pollen presence on the oviposition behaviour of the monarch butterfly, *D. plexippus*, was assessed in cage and flight chamber studies. The proportions of monarch eggs oviposited on milkweed plants dusted with Bt pollen, and untransformed hybrid, gravel dust, and undusted control plants were recorded from a cage study. None of the treatments differed significantly in the relative proportion of eggs found. The effect of Bt and non Bt corn plant proximity and corn pollen presence was also assessed in a flight chamber. A significantly higher proportion of eggs (96%) were recovered from patches of milkweed plants not surrounded by corn plants, and a significantly higher proportion of eggs (nearly 70%) were recovered from patches of milkweed plants not dusted with corn pollen. It was concluded, that "there were no significant differences in the effects of Bt corn plants or corn pollen compared with non Bt plants or pollen" (Tschenn *et al* 2001). Moreover, in the following detailed study of Bt corn pollen risk to monarch butterfly *D. plexippus* (L.) populations, the results were compared with more highly refined risk assessment techniques in terms of the risk conclusions which can be developed with more highly certain information. Exposure analysis showed pollen interception by the host for monarch butterfly larvae (common milkweed, *Asclepias syriaca* L.) declined exponentially with distance from the pollen source. Intra- and inter-genera sensitivity of lepidopteran species was used to project effect to monarch butterfly larvae. When the 90(th) percentile of effective (LC_{50}) was used to estimate monarch butterfly sensitivity to Bt corn pollen expressing Cry1A (b) toxin, "the risk of lethality to individual larvae was negligible" (Wolt *et al* 2003).

Bt corn expressing Cry3Bb1 toxin was developed for control of corn rootworms, *Diabrotica* spp. (Coleoptera: Chrysomelidae). A continuous feeding study was conducted in the laboratory to evaluate the dietary effect of the pollen expressing the Cry3Bb1 protein on the survival, larval development, and reproductive capacity of the nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Results demonstrated that when offered at 50% by weight of the dietary component, Bt corn pollen expressing Cry3Bb1 protein had no measurable negative effect on the survival and development of *C. maculata* larvae to pupation and adulthood nor any adverse effect on adult survival and reproductive capacity (Duan *et al* 2002).

An assessment on the impact of *Bt* cotton pollen on two important economic insects, the Chinese ussah silkworm, *Antnaea perngicuerin* (*Antheraea pernyi*) and the silkworm, *Bombyx mori*, was conducted, it was concluded that the adverse effect is negligible (Wu *et al* 2003).

PI protected TPs: For testing the effects of protease inhibitor-expressing crops on nontarget herbivorous insects a sequential approach has been developed. The approach consisted of five tiers. In tier 1, field surveys was used to characterize the nontarget invertebrate fauna of a crop. In tier 2, histochemical assays was used to identify the subset of herbivores with a particular class of digestive proteolytic enzymes. In the assessment phase a combination of laboratory 'worst-case scenario' studies (tier 3) and controlled environment or small-scale field trials (tier 4) were used to evaluate the impact of the protease inhibitor-expressing plants on the selected nontarget species. In the final tier, field trials were used to compare the relative effect of transgenic plants and current management practices, such as pesticide use, on selected species. The first four tiers of the approach were described using transgenic potatoes expressing cystatins, a family of cysteine proteinase inhibitors, as an example. Although the TPs had enhanced levels of resistance to potato cyst nematodes (PCN), *Globodera pallida* and *Globodera rostochiensis*, the results established that they have negligible impact on the nontarget herbivorous insect, *Eupteryx aurata* (Cowgill and Atkinson 2003).

In general, the extensive testing on nontarget plant-feeding insects and beneficial species that has accompanied the long-term and wide-scale use of Bt plants has not detected significant adverse effects (reviewed in O'Callaghan *et al* 2005).

2.1 *Will insecticidal TPs damage arthropods, especially, honey bees?*

Species representing pollinators, natural enemies (including predators and parasitoids), and detritovores are among the insects that have been subjected to tests with TPs. Insects without obvious ecological roles but which are considered representative of valued biodiversity (e.g. endangered or culturally valued species) have also been studied. Among the many insect pollinators of agricultural crops, honey bees are the best known. Neither Bt corn nor Bt cotton requires bees for pollination, but cotton nectar is attractive to them and produces a useful honey, corn pollen may be collected when other pollen sources are scarce

(i) *Yes*

Honey bees are obvious non-target arthropods to be included in a risk assessment procedure but due to their complex social behaviour, testing transgene products on individual bees is not possible in bee colonies. A laboratory larval rearing technique was employed to test the impacts of such transgene products on honey bees *Apis mellifera* L. A serine proteinase inhibitor (Kunitz soybean

trypsin inhibitor, SBTI), that is a source of insect resistance in TPs, was used as a model insecticidal protein on honey bee larvae reared individually in the laboratory. The addition of 1·0% SBTI (w : w of total protein) to the larval diet created significant additional larval mortality, slowed juvenile development and significantly decreased adult body mass (Burgess *et al* 1996; Brodsgaard *et al* 2003).

Also, it was shown, that PI can affect bumble bees causing changes in bee digestive proteases and some reductions in survival when ingested at high concentrations (Malone *et al* 2000).

(ii) No

The impact of transgenic oilseed rape (*Brassica napus* L.) on the foraging behaviour of honey bees (*A. mellifera* L.) was evaluated for two different lines expressing constitutively heterologous chitinase in somatic tissue for enhanced disease resistance. Experiments were conducted in confinement in an indoor flight room with controlled conditions and in an outdoor night cage with conditions more representative of the open environment. Foraging behaviour was analysed by observations of general bee behaviour (total number of visits) and of individual bee behaviour (using a video camera coupled with a special software program to process the data). The results showed no effects on bee foraging behaviour due to the TPs (Picardnizou *et al* 1995). Pre-release honey bee biosafety tests have been conducted for each Bt crop registered in the United States, including Cry9C corn and Cry3A potatoes. Each test involved feeding bee larvae and sometimes adults with purified Cry proteins in sucrose solutions at concentrations that greatly exceeded those recorded from the pollen or nectar of the TPs in question. In each case, no effects were observed (United States Environmental Protection Agency 2000).

Transgenic strawberry lines expressing the cowpea trypsin inhibitor (CpTi) were examined under field conditions to determine if this gene offered protection against the feeding of vine weevil (*Otiorhynchus sulcatus* F.). Field results for two years demonstrated protection in terms of attack by vine weevil larvae on all the transgenic plants. The CpTi has a significant effect on vine weevil in terms of a reduction in the number of pupae. The numbers of Carabid and other non-target arthropods were assessed over the duration of the trial, and were found not affected significantly by the CpTi TPs (Graham *et al* 2002).

The potential impact of biotin-binding proteins (BBPs) on honey bees was studied and the effects of feeding a purified BBP, avidin, to honey bee larvae and adults were determined. A realistic larval dosing regime was developed by estimating the pollen content of brood food in the field and adding avidin to artificial diet at rates that simulated the presence of avidin-expressing transgenic pollen in brood food. Larval survival and development were unaffected by avidin in assays which simulated larvae receiving pollen expressing 0, 4 or 40 μM avidin at concentrations of 164 μg pollen per mg food for the first 2 days and 880 μg pollen per mg food thereafter. Food consumption and survival of adult bees were also unaffected by avidin added to pollen-candy at levels corresponding to pollen expression of 0, 6·7 or 20 μM avidin (Malone *et al* 2002).

2.2 *Will herbicide tolerant plants adversely affect biodiversity?*

Glyphosate-resistant soybean *Glycine max* (L.) Merr expressing an insensitive 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) gene has revolutionized weed control in soybean production. Of the TPs currently in commercial use, glyphosate-resistant soybeans have probably received the most attention from both proponents and opponents of TPs. Transgenic soybeans are an example of an input-substituting technological innovation. Adoption is a private decision, based on the adopter's assessment of private costs and benefits, but may also have external effects. Are there any evidences, that they could be clearly negative?

(i) *No*

The effects of transgenic soybean varieties and their corresponding weed management strategies on canopy insects were examined in studies at two locations in Iowa (USA) in 1997 and 1998. Weed management systems that allowed more weed escapes typically had higher insect population densities. However, systems with fewer weeds seemingly were preferred by potato leafhoppers. Bean leaf beetles and potato leafhoppers showed preferences for certain soybean varieties. but these effects were attributed to soybean plant height. It was concluded, that although the HT soybean varieties did not strongly affect insect populations, weed management systems can affect insect populations in soybean. However, this impact was likely related more to weed suppression effectiveness than to a direct effect of the herbicides on the insects (Buckelew *et al* 2000).

The degree of abundance and diversity of springtails (order Collembola) often indicates the extent of disturbance by various agricultural practices. It was examined how HT soybean varieties and their associated weed management systems affect the abundance of 21 surface-active springtail species during three successive soybean growing seasons. With six soybean varieties (three transgenic, three nontransgenic), three weed management sys-

tems were tested: (i) targeted application of specific herbicides to the corresponding HT transgenic varieties; (ii) conventional pre-and postemergence herbicide applications; and (iii) mechanical cultivation. Each method posed its own potential costs and benefits to springtails. In targeted plots, springtail numbers were similar to or higher than those in conventional plots, suggesting that the later and repeated targeted applications to transgenic soybeans do not adversely affect springtail numbers in the short term. The observed treatment effect differences was attributed on springtail numbers to resultant differences in weed cover and degree of soil disturbance (indirect effects), rather than to any direct toxic effects of the herbicides. The treatments affected some species but not others; most of the affected species responded similarly to differences in weed treatment. The results overall suggested no deleterious short-term effects of HT soybean targeted weed-management systems on abundance of the springtail species examined (Bitzer *et al* 2002).

2.3 *Will insecticidal TPs damage food chains?*

Predators and parasitoids are significant regulators of insect pest populations, and integrated pest management systems strive to harmonize pesticide use with the preservation or augmentation of these natural enemies. There are two approaches to the utilization of natural enemies of insect pests in an agricultural system: preservation and augmentation of existing predators and parasites, and mass rearing of natural enemies for release to regulate the population density of the target insect pest. In recent years there have been many studies on potential tritrophic impacts of TPs as well as field surveys of natural enemy abundance on TPs. Natural enemy survival depends upon a supply of host insects, so reductions in host numbers feeding on TPs plants will affect population densities of natural enemies, as will any pest control measure. In addition, TPs could have a direct 'prey-mediated' effect on individual natural enemies via ingestion of transgenic pollen, other plant tissue, or active recombinant protein in the bodies of their prey. Indirect effects could also result from prey being smaller, sicker, or less palatable for having fed on the TPs (reveiwed in Astrid *et al* 2002). As food web components, it is reasonable to distinguish; (i) target herbivores; (ii) non-target herbivores; (iii) pollinators; (iv) parasitoids; and (v) predators.

So, are there any evidences, that TPs will negatively affect at least one of these components?

(i) *Yes*

Bt protected corn: In laboratory feeding experiments Bt corn have been carried out to study the effects of Bt fed herbivores (i.e. prey) on *Chrysoperla carnea* Stephens. (the green lacewing an important predatory insect in corn). Host plants were Bt corn expressing CrylAb toxin and the corresponding non Bt corn. Two different preys were used: the European corn borer, *Ostrinia nubilalis* (Hubner) (lepidopterous target pest), and *Spodoptera littoralis* (Boisduval) (lepidopterous nontarget pest for *B. thuringiensis*). The objectives were to quantify the effects of Bt corn-fed prey on chrysopid immature development and to determine whether observed effects were caused by sick, suboptimal prey (indirect effects) or associated with Bt corn related causes (direct effects). Mean total immature mortality for chrysopid larvae raised on Bt corn-fed prey was 62% compared with 37% when raised on non Bt corn prey. There was no significant difference in mortality between chrysopid larvae reared on Bt corn fed *O. nubilalis* or Bt corn-fed *S. littoralis*. Similarly, no significant difference in mortality was detected when chrysopid larvae were raised on non Bt corn *O. nubilalis* or non Bt corn *S. littoralis*. Development time of chrysopid larvae was prolonged when Bt corn-fed *O. nubilalis* was given to the predators but not for Bt corn-fed *S. littoralis*. Although some unnoticed adverse effects in *S. littoralis* may have occurred because of the Bt corn, the results suggested that the reduced fitness of chrysopid larvae was associated with Bt corn. The prolonged development time of chrysopid larvae raised on Bt corn-fed *O. nubilalis* was probably because of a combined effect of BT toxin exposure and nutritional deficiency caused by sick prey (Hilbeck *et al* 1998). In a multiple years research project, tritrophic and bitrophic effects of Bt corn expressing Cry1Ab toxin on the natural enemy species, *C. carnea*, was investigated. And prey-mediated effects of Bt-corn causing significantly higher mortality of *C. carnea* larvae was observed. In further laboratory trials it was shown that the route of exposure (fed directly or via a herbivorous prey) and the origin of the Bt (from transgenic plants or incorporated into artificial diet) strongly influenced the degree of mortality. In choice feeding trials where *C. carnea* could choose between *S. littoralis* fed Bt-corn and *S. littoralis* fed non-Bt corn, larger instars showed a significant preference for *S. littoralis* fed non-Bt corn while this was not the case when the choice was between Bt- and isogenic corn fed aphids (Hilbeck 2001).

Field surveys, which did not distinguish population-level, prey-mediated, or direct effects, have shown little impact of Bt corn on predator species numbers or densities. Of nine predator species (including green lacewings), only *Coleomegilla* or *Chrysopa maculata* larvae were found at significantly lower densities on Bt sweet corn (Wold *et al* 2001).

To assess the ecological effects of Bt-corn, expressing the Cry1Ab toxin, on larvae of *C. carnea*, the following

factors were examined: (i) the performance of three prey herbivores (*Rhopalosiphum padi*, *Tetranychus urticae*, and *Spodoptera littoralis*) on transgenic Bt and non-transgenic corn plants; (ii) the intake of the Cry1Ab toxin by the three herbivores; and (iii) the effects on *C. carnea* when fed each of the prey species. A higher mortality rate and a delay in development were observed in *S. littoralis* larvae when fed Bt-corn compared with those fed the control corn plants. The ingestion of Cry1Ab toxin by the different herbivores was measured using an immunological assay (ELISA). Highest amounts of Cry1Ab toxin were detected in *T. urticae*, followed by *S. littoralis*, and only trace amounts detected in *R. padi*. Feeding *C. carnea* with *T. urticae*, which were shown to contain the Cry1Ab toxin, or with *R. padi*, which do not ingest the toxin, did not affect survival, development, or weight of *C. carnea*. In contrast, a significant increase in mortality and a delay in development were observed when predators were fed *S. littoralis* larvae reared on Bt-corn. It was suggested, that a combined interaction of poor prey quality and Cry1Ab toxin may account for the negative effects observed on *C. carnea* when fed *S. littoralis* (Dutton *et al* 2002).

Another study was performed to evaluate the effects on various fitness parameters in parasitoids that develop on intoxicated hosts. The parasitoid used in this study was *Parallorhogas pyralophagus* (Marsh), a gregarious, external idiobiont, and the host was *Eoreuma loftini* Dyar, a subtropical stemborer. Results showed that ingestion of Bt corn (Cry1Ab) tissue by *E. loftini* larvae negatively affected some fitness components in *P. pyralophagus*, whereas other components were not affected. Specifically, immature stage developmental mortality was greater, adult longevity was approximately 1 day shorter in females, and developmental times were approximately 2 day longer in both males and females when *P. pyralophagus* developed on hosts fed Bt corn tissue relative to hosts fed non-Bt corn tissue. Moreover, parasitoid brood size was positively correlated with host size when hosts were fed non-Bt corn tissue, while this relationship was absent when hosts were fed Bt corn tissue. However, feeding on Bt corn tissue did not affect egg loads, adult size-egg load and adult size-longevity relationships, adult sizes of females and males, and sex ratio of parasitoids whose hosts were fed Bt corn tissue (Bernal *et al* 2002).

The ecological assessment of the effects of Dipel, one of the most widely used Bt-sprays in agriculture, on *C. carnea* larvae was made. Indirect effects due to a reduction of prey were tested by rearing three prey species of *C. carnea* (the aphid *R. padi*, the spider mite *T. urticae*, and Lepidoptera larva *S. littoralis*) on either corn plants sprayed with Dipel (at the recommended field concentrations) or on control plants. Effects of Dipel on *C. carnea* were assessed by performing greenhouse experiments in which chrysopid larvae were kept on Bt-sprayed or control plants and fed with herbivores reared on Bt-sprayed or control plants. Dipel had no effect on aphids; however, negative effects on spider mites were observed. Spider mites reared on Bt-sprayed plants had a significantly lower intrinsic rate of natural increase compared to those reared on control plants. Similarly, *S. littoralis* larvae were significantly affected by Dipel as the developmental time required by larvae which were fed Bt-sprayed plants was prolonged when compared to larvae on untreated plants. Negative effects on *C. carnea* larvae were also shown through prey-mediated exposure to Dipel. A significant increase in mortality, a prolonged developmental time and a slight decrease in weight was observed for *C. carnea* fed with 'Bt-contaminated' *S. littoralis* larvae (Dutton *et al* 2003a,b).

Bt protected cotton: Potential negative tritrophic impacts of Bt protected cotton (Cry1Ac) on the predators *Orius tristicolor* and *Geocoris punctipes* (but not on *Nabis* spp. or *Zelus renardii*) (Ponsard *et al* 2002) and on the parasitoids *Cotesia marginiventris* and *Copidosoma floridanum* (Baur and Boethel 2003) have been demonstrated in laboratory studies.

Lectin protected plants: The snowdrop lectin, *Galanthus nivalis* agglutinin (GNA), has been the subject of many natural enemy studies. In fact, some negative effects have been observed, although the impacts in field situations have not yet been ascertained. Experiments using prey fed artificial GNA diets have not always produced results consistent with those using GNA-expressing plants.

GNA provide partial resistance to two potato aphids *Myzus persicae* and *Aulacorthum solani*, when incorporated in artificial diet and/or expressed in transgenic potato. Trangenic potato line expressing same anti-aphid plant GNA protein, was assessed for possible influence of the insecticidal toxicity on a beneficial pest predator and to demonstrate adverse tri-trophic interactions involving a lectin-expressing transgenic crop, a target pest aphid and a beneficial aphidophagous predator. When adult 2-spot ladybirds (*Adalia bipunctata* L.) were fed for 12 days on peach-potato aphids (*Myzus persicae* Sulzer) colonizing transgenic potatoes expressing GNA in leaves, ladybird fecundity, egg viability and longevity significantly decreased over the following 2–3 weeks. No acute toxicity due to the TPs was observed, although female ladybird longevity was reduced by up to 51%. Adverse effects on ladybird reproduction, caused by eating peach-potato aphids from transgenic potatoes, were reversed after switching ladybirds to feeding on pea aphids from non-transgenic bean plants. These results demonstrated that expression of a lectin gene for insect resistance in a transgenic potato line can cause adverse effects to a pre-

datory ladybird via aphids in its food chain (Birch *et al* 1999). Dose-dependent effects on the development of the aphid parasitoid *Aphidius ervi* were noted when parasitoids were fed *M. persicae* aphids raised on a GNA-containing diet, but not aphids raised on GNA potatoes (Couty *et al* 2001a,b).

First-tier laboratory-scale experiments were conducted to assess the potential effect of GNA on the aphid parasitoid *Aphelinus abdominalis*. GNA (0·1% w/v) was successfully delivered to *Macrosiphum euphorbiae* via artificial diet and induced a reduced growth rate and increased mortality compared to aphids fed a control diet. As aphid parasitoid larvae are endophagous, they may be exposed to GNA during their larval development and potential 'chronic toxicity' on *A. abdominalis* was investigated. Results suggested that parasitoids excrete most of the GNA ingested. Sublethal effects of GNA on several parasitoid fitness parameters (parasitism success, parasitoid development and size, emergence success, progeny survival and sex ratio) were studied. No direct detrimental effect of GNA on *A. abdominalis* was observed. However, GNA had an indirect host-size-mediated effect on the sex ratio and the size of parasitoids developing in GNA-fed aphids. This work highlighted the need to determine the exact 'causes and effects' when assessing the ecological impact of TPs on non-target beneficial insects (Couty *et al* 2001a,b). GNA-potato-fed prey had a negative effect on egg viability of the lady beetle *Adalia bipunctata*, but prey fed a GNA diet increased the fertility of this predator (Down *et al* 2003). Prey that were fed GNA diets or GNA potato were less favoured or resulted in smaller, shorter-lived adult parasitoids *P. pyralophagus* (Tomov *et al* 2003). The parasitoids *Aphidius colemani*, *Trichogramma brassicae*, and *Cotesia glomerata* had reduced longevity when fed sucrose solutions containing GNA (Romeis *et al* 2003a). Negative effects have been recorded for the predator *P. maculiventris* on tomato moth caterpillars injected with GNA (reduced growth) or fed GNA potato (reduced fecundity) (Bell *et al* 2003).

Transgenic wheat plants expressing GNA under the control of constitutive and phloem-specific promoters were tested for enhanced resistance to the grain aphid (*Sitobion avenae*) by exposing the plants to nymphal insects under glasshouse conditions. Bioassay results demonstrated that transgenic wheat plants from lines expressing GNA at levels greater than ca. 0·04% of total soluble protein decrease the fecundity, but not the survival, of grain aphids (Stoger *et al* 1999).

Cotesia flavipes (Cameron) is a parasitoid responsible for maintaining populations of sugarcane borer, *Diatraea saccharalis* (F.), below economic levels in south Texas sugarcane fields. Transgenic sugarcane expressing GNA provide resistance against the Mexican rice borer, *Eoreuma loftini* (Dyar), the primary pest of south Texas sugarcane. The potential impact of GNA-expressing sugarcane on various biological and fitness parameters of *Cotesia flavipes* (Cameron) was studied in the laboratory to gain insight on likely effects of the transgenic sugarcane on biological control of sugarcane borer by *C. flavipes*. Females of *C. flavipes* were offered sugarcane borer larvae fed one of two diet treatments for oviposition for two successive generations: (i) artificial diet containing transgenic sugarcane tissue or (ii) artificial diet containing nontransgenic sugarcane tissue. Small to marginal negative effects of artificial diet containing transgenic sugarcane tissue were evident in the rate of host suitability, number of cocoons and adult parasitoids emerging per host, percentage cocoons yielding parasitoids, and sex ratio and adult lifespan of parasitoids. These effects were variable between the two parasitoid generations examined. In contrast, differences were not detected between diet treatments in rates of host acceptance, egg load of fentales, and egg to adult developmental periods. The negative effects of transgenic sugarcane on *C. flavipes* detected in this study was important because GNA levels in the diet (approximate to 0·49% of total protein content) containing transgenic sugarcane tissue were approximate to 50% of the level expressed in transgenic sugarcane plants (Setamou *et al* 2002).

PI protected plants: Parasitoids, *Eulophus pennicornis*, were adversely affected by both CpTI injected and CpTI-potato-fed *L. oleracea* prey (Bell *et al* 2001). The potential prey-mediated impacts of TPs expressing proteinase inhibitor (PI) on the predatory carabid, *Nebria brevicollis* non-target impacts were investigated. The PI used was aprotinin, a serine PI of mammalian origin with insecticidal properties when incorporated in artificial diet or expressed in TPs. Field-collected *N. brevicollis* adults were fed, over their pre-aestivation activity period of 24 days, with *Helicoverpa armigera* larvae reared on an artificial diet containing 0·5% (w/w, fresh mass) aprotinin. These larvae contained 22–62 μg aprotinin/g insect. Control prey was reared on diet without aprotinin. Beetle survival and body mass were unaffected by prey type. Beetles consuming PI-fed prey lost significantly more mass than the control beetles during two periods of mass loss, but gained significantly more mass during the final period of mass gain. This was not due to differences in amounts of prey supplied or consumed. The final mass gain coincided with increased consumption of PI-prey. Female beetles were significantly heavier than males, but no consistent gender-based differences in response to PI-prey was found. At the end of the experiment, body mass of all beetles was similar to field-collected ones (approximately 55 mg). All experimental beetles had significantly lower activities of digestive cysteine proteases and

the serine proteases chymotrypsin and trypsin than field-collected ones. Beetles consuming PI-fed prey had significantly lower levels of trypsin and higher levels of chymotrypsin and elastase than the control beetles (Burgess *et al* 2002).

The effects of oilseed rape expressing the serine protease inhibitor, mustard trypsin inhibitor-2 (MTI-2), on the predatory ground beetle *Pterostichus madidus* were investigated, using diamondback moth, *Plutella xylostella* as the intermediary pest species. As expected, oilseed rape expressing MTI-2 had a deleterious effect on the development and survival of the pest. However, incomplete pest mortality resulted in survivors being available to predators at the next trophic level, and inhibition studies confirmed the presence of biologically active transgene product in pest larvae. Characterization of proteolytic digestive enzymes of *P. madidus* demonstrated that adults utilize serine proteases with trypsin-like and chymotrypsin-like activities (Ferry *et al* 2005).

(ii) *No*

Bt protected corn: Laboratory studies determined the effects of feeding Bt corn pollen expressing a CrylAb toxin on 3 predatory species: *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), *Orius insidiosus* Say (Heteroptera: Anthocoridae), and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). No acute detrimental effects of the Bt pollen on preimaginal development and survival were observed among these predators. Moreover, no detrimental effects were observed in the abundance of *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) predators (coccinellids, anthocorids, chrysopids) in Bt corn compared with non-Bt corn during 2 years of field evaluations. Predator numbers observed before, during, and after pollen shed suggested that Bt corn pollen will not affect natural enemy movement in corn (Pilcher *et al* 1997).

Laboratory feeding experiments with Bt corn (Cry1Ab) were carried out to study the effects Bt corn-fed herbivorous prey on the predator *Orius majusculus* (Reuter). The herbivorous prey species used was *Anaphothrips obscurus* (Muller), a thysanopteran pest of corn, not sensitive to Cry1Ab toxin. The objectives were to quantify the effects of Bt corn-fed prey on the development and mortality of immature *O. majusculus*. There was no significant difference in total mean mortality from hatch to adult eclosion between *O. majusculus* nymphs reared on Bt corn-fed or non Bt corn fed prey. Similarly, no significant differences in total developmental time of *O. majusculus* were detected when reared on the two different prey types. Overall mortality was low, confirming that the methodology used was appropriate (Zwahlen *et al* 2000).

Phloem sap of Bt corn expressing a truncated form of Cry1Ab, toxin, sap sucking aphids feeding on Bt corn and their honeydew were analysed for the presence of Cry1Ab using ELISA. No Cry1Ab was detected in the phloem sap. In contrast, measurable concentrations of Cry1Ab in the range of 1 ppb were detected when phloem sap of pooled leaf samples was extracted using EDTA buffer. This was probably because of Cry1Ab toxin released from damaged cells. When analysing apterous adults of *R. padi* L. and their honeydew, no Cry1Ab could be detected. In contrast, Cry1Ab was clearly detected in both larvae of the leaf chewing herbivore *S. littoralis* (Boisduval) and their faeces, showing that Cry1Ab is detectable after ingestion and excretion by herbivores. These results suggested that *R. padi* ingests or contains no or only very low concentrations of Cry1Ab in the range of the detection limit. In consequence it was proposed that *R. padi* as an important prey for beneficial insects in corn is unlikely to cause any harm to its antagonists due to mediating Bt toxin (Raps *et al* 2001).

Subsequent tritrophic studies with several different Cry1Ab corn fed prey species and the green lacewing confirmed that the toxin itself does not affected this predator but that the suboptimal quality of Bt-corn-susceptible prey species may have an impact. When given a two-way choice, the lacewings preferred non-Bt-corn-fed *S. littoralis* caterpillars to Bt-fed larvae (Meier and Hilbeck 2001).

A laboratory experiment examining the effect of two different Bt corn tissue on *Euchatias egle* Drury (Lepidoptera: Arctiidae) showed no larval mortality following a 48 h exposure to Bt corn tissues on its food source, *Asclepias syriaca* (Asclepiadacea). One of 15 larvae exposed to non-Bt corn tissue died compared to no mortality of the larvae exposed to either Bt11 or 176 Bt corn tissues. Based on these results it was suggested that *E. egle* will not be adversely affected by the wide-scale planting of Bt corn (Jesse and Obrycki 2002). Consuming *Tetranychus urticae* mites, which were not Bt susceptible but carried measurable quantities of Cry1Ab after sucking the cell contents of Bt corn had no effect on green lacewings (Dutton *et al* 2002). The lady beetle and 11 other natural enemy species or groups of species (including a *Chrysopa* sp.) were not affected by Bt corn in a recent Ohio study (Jasinski *et al* 2003). The predators *C. maculata*, *Harmonia axyridis*, and *Orius insidiosus* were less affected by Bt sweet corn than by pyrethroid sprays (Musser and Shelton 2003). A recent study in which green lacewings were fed high doses of Cry1Ab toxin showed no negative impacts and confirmed that negative tritrophic effects observed with this predator and some prey fed Bt corn were entirely prey mediated (Romeis *et al* 2004).

Bt protected rice: Five Bt rice lines (Cry1Ab, Cry1Ac) were tested for effects on two non-target insects: the

brown planthopper, *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae), and its predator *Cyrtorhinus lividipennis* (Hemiptera: Miridae). Bt toxin was detected by ELISA in the honeydew of *N. lugens* that fed on Bt rice plants. The amount of honeydew derived from phloem feeding did not differed between Bt and control lines. There were no differences between *N. lugens* reared on Bt and control lines in any of the five fitness parameters measured (survival to the adult stage, male and female weight, and male and female developmental time). In general these detailed study indicated that *N. lugens* and its natural enemies in fact will be exposed to Bt toxins from Bt rice but that this exposure might not affect *N. lugens* and *C. lividipennis* fitness (Bernal *et al* 2002). Field tests with rice expressing Cry1Ab and Cry1Ac showed no effects on the population dynamics of five spider species (Liu *et al* 2002).

Bt protected cotton: *Bt* cotton has been widely planted in China since 1997. Results obtained in recent years indicated that the predator levels in *Bt* cotton fields are significantly higher than those in conventional cotton fields where insecticide has been used for control of the cotton bollworm, *Helicoverpa armigera*. However, the population density of parasitic wasps which parasitise on *H. armigera* larvae decreases dramatically due to the lower density and poor quality of *H. armigera* larvae in *Bt* cotton fields. As the predator population increases, the outbreak of cotton aphid in mid-season is effectively controlled, while the mirids become key insect pests in *Bt* cotton fields because of a reduced number of insecticides used against *H. armigera*. The diversity of the arthropod community in *Bt* cotton fields was higher than that in conventional cotton, suggesting that *Bt* cotton is highly favourable for integrated management of cotton pests (Wu *et al* 2003).

Bt protected potato: The detailed field studies were conducted in 1992 and 1993 in Hermiston, Oregon, to evaluate the efficacy of Bt potato expressing Cry3Aa toxin and conventional insecticide spray programs against the important potato pest, *Leptinotarsa decemlineata* (Say), Colorado potato beetle (CPB), and their relative impact on non-target arthropods in potato ecosystems. Results from the two years of field trials demonstrated that Bt plants were highly effective in suppressing populations of CPB, and provided better CPB control than weekly sprays of a microbial Bt-based formulation containing Cry3Aa. When compared with non Bt potato plants not treated with any insecticides, the effective control of CPB by Bt potato plants or sprays of a Bt-based formulation did not significantly impact the abundance of beneficial predators or secondary potato pests. In contrast to Bt plants or microbial Bt formulations, however, applications of permethrin significantly reduced the abundance of several major generalist predators such as spiders (Araneae), big-eyed bugs (*Geocorus* sp.), damsel bugs (*Nabid* sp.), and minute pirate bugs (*Orius* sp.), and resulted in significant increases in the abundance of green peach aphid (GPA), *Myzus persicae* (Sulzer), vector of viral diseases, on the treated potato plots. While systemic insecticides appeared to have reduced the abundance of some plant sap-feeding insects such as GPA, lygus bugs, and leafhoppers, early and mid-season applications of these insecticides had no significant impact on populations of the major beneficial predators (Reed *et al* 2001).

The effect of Bt potatoes expressing (Cry3A) toxin insceticidal to the CPB was studied on the season-long relative abundance of naturally-occurring generalist predators. Low inputs of foliar insecticides were used in the transgenic fields to suppress nontarget pests and in the nontransgenic fields to prevent total defoliation of potato plants by *L. decemlineata*. Dominant plant-foraging heteropteran predators and lady beetles were sampled by sweeping foliage, whereas, ground-foraging carnivorous carabids, ants, and spiders were sampled by trapping in pitfalls. *Orius insidiosus* (Say) was significantly more abundant in transgenic fields than in non transgenic fields in 1994, but not in 1995. None of the coccinellids (3 taxa) were affected by the treatments in either season. The carnivorous carabids (3 taxa) and ants were not affected by either treatment, but spiders were significantly more abundant in the transgenic fields in 1995. It was concluded, that the deployment of pure stands of Bt potatoes (Cry3A), with a minimum input of insecticides to suppress non-target pests, will have no deleterious effects on the populations of generalist predators in the potato ecosystem (Riddick *et al* 2000).

Other Bt protected TPs: Two lines of Bt oilseed rape (*Brassica napus*) were tested for side-effects on the hymenopteran parasitoid *Diaeretiella rapae* and its aphid host, *Myzus persicae*. One transgenic line expressed the F-endotoxin Cry1Ac (Bt) and a second expressed the proteinase inhibitor oryzacystatin I (OC-I) from rice. These transgenic plants were developed to provide resistance to lepidopteran and coleopteran pests, respectively. No detrimental effects of the transgenic oilseed rape lines on the ability of the parasitoid to control aphid populations were observed. Adult parasitoid emergence and sex ratio were also not consistently altered on the transgenic oilseed rape lines compared with the wild-type lines (Schuler *et al* 2001).

The diamondback moth (*Plutella xylostella*), a major pest of brassica crops, is normally highly susceptible to a range of Bt toxins. However, extensive use of microbial Bt sprays has led to the selection of resistance to Bt tox-

ins in *P. xylostella*. *Cotesia plutellae* is an important endoparasitoid of *P. xylostella* larvae. Although unable to survive in Bt-susceptible *P. xylostella* larvae on highly resistant Bt oilseed rape plants due to premature host mortality, *C. plutellae* is able to complete its larval development in Bt-resistant *P. xylostella* larvae. Experiments of parasitoid flight and foraging behaviour showed that adult *C. plutellae* females do not distinguish between Bt and wildtype oilseed rape plants, and are more attracted to Bt plants damaged by Bt-resistant hosts than by susceptible hosts. This stronger attraction to Bt plants damaged by resistant hosts was due to more extensive feeding damage. Population scale experiments with mixtures of Bt and wildtype plants demonstrated that the parasitoid is as effective in controlling Bt-resistant *P. xylostella* larvae on Bt plants as on wildtype plants. In these experiments equal or higher numbers of parasitoid adults emerged per transgenic as per wildtype plant (Schuler *et al* 2003).

Lectin protected TPs: Tritrophic interactions between transgenic potato expressing GNA an aphid pest, *Myzus persicae* (*Sulz.*), and a beneficial predator, the 2-spot ladybird (*Adalia bipunctata* L.) were investigated. Clonal plants expressing GNA at 0·1–0·2% total soluble protein in leaves were used. No significant effects on development and survival of ladybird larvae fed on aphids from these transgenic plants were observed, with larval survival in the experimental group being 90% compared to 89% for controls. There were also no effects on subsequent female or male longevity. Female fecundity was also investigated. Although no significant differences were observed in egg production between control and experimental groups, a 10%, reduction in egg viability (determined by percent hatch) occurred in ladybirds fed aphids reared on transgenic plants. The special studies were carried out using aphids fed on artificial diet containing GNA, to deliver quantified levels of the protein to ladybird adults. GNA had no deleterious effects upon adult longevity, but resulted in a consistent trend for improved fecundity. Egg production was increased by up to 70% and egg viability also increased significantly. The results suggested that GNA was not deleterious to ladybirds (Down *et al* 2003).

PI protected TPs: Cysteine proteinase inhibitors (cystatins) confer resistance to plant-parasitic nematodes when expressed in TPs. The survival and growth of nymphs of the peach-potato aphid, *Myzus persicae*, were adversely affected when cystatins were added to artificial diets. When aphids were clip-caged onto transgenic plants expressing chicken egg white cystatin (CEWc) there was no adverse effect on aphid fitness. Field populations of aphids on transgenic Desiree potatoes, expressing CEWc or a modified version of oryzacystatin I, were not significantly different from populations on control non transgenic Desiree plants (Cowgill *et al* 2002). CpTi-injected tomato moth caterpillars (*Lacanobia oleracea*) given to the predator *Podisus maculiventris* resulted in reduced nymph growth and adult female weights, but experiments with the same insects fed CpTi potatoes showed no negative effects on the predator (Bell *et al* 2003). Stinkbug predators (*Perillus bioculatus*) feeding on CPB reared on OCI potatoes compensate for any effects of this PI by upregulation of their digestive proteases (Bouchard *et al* 2003a,b).

In general Bt plants that are insecticidal only to selected groups of insect species (e.g. Lepidoptera) are unlikely to have direct effects on natural enemy species outside this group. Transgenic proteins with ranges of activity wider than those of Bt may have a greater chance of affecting natural enemies, but research with PIs and lectins so far has suggested that these effects are not major. There is no evidence that insecticidal proteins accumulate along food chains; in fact, the available data report dilution instead (O'Callaghan *et al* 2005).

3. Will Bt toxin produced by TPs be active in a soil?

An obvious concern is that increased levels of insecticidal proteins produced by TPs could damage beneficial microorganisms such as mycorrhizae, rhizobia and other organisms involved in plant health, litter decomposition and nutrient cycling. The potential impacts of TPs on soil organisms depend, at least in part, on the persistence of the transgene-derived protein and its biological activity in soil. Bt endotoxin from corn enters the soil from both root exudates (Saxena 1999, 2002) and postharvest residues (Saxena and Stotzky 2001a). So, are there evidences of an accumulation of TPs expressed trangenic proteins in soil?

(i) *Yes*

Bt protected TPs: The equilibrium adsorption and binding of the active toxin from *B. thuringiensis* subsp, kurstaki, toxic to lepidopteran larvae, to humic acids extracted from two forest and two cultivated soils, as well as the insecticidal activity and the biodegradation of the bound toxin. were studied. From 75 to 85% of the toxin added was rapidly adsorbed to the humic acids at equilibrium, and adsorption to a constant amount of humic acids increased with the concentration of the toxin until a plateau was reached. Differences in total acidity and in the content of phenolic groups of the humic acids appeared to be primarily responsible for differences in the amounts of toxin bound (45–80% of the adsorbed toxin) after extensive washing with distilled water. Bound humic acid-

toxin complexes were toxic to larvae of the tobacco hornworm (*Manduca sexta*). The lethal concentration necessary to kill 50% of the larvae (LC_{50}) of the bound toxin was comparable with that of the free toxin, indicating that the binding of the toxin to humic acids did not affect its insecticidal activity. The bound toxin did not support the growth of a mixed microbial culture from soil, although the free toxin was rapidly utilized as a carbon and energy source for growth, indicating that binding of the toxin to humic acids reduced its biodegradability. These results indicated that the toxins from *B. thuringiensis* expressing by TPs and microbes could persist, accumulate, and remain insecticidal in soil as a result of binding to humic acids, as well as on clays (Crecchio and Stotzky 1998). In the next study it was shown that Bt toxins produced by various subspecies of *B. thuringiensis* and bacterial transforming DNA bind rapidly and tightly on clays, both pure mined clay minerals and soil clays and on humic acids extracted from soil. This binding reduces the susceptibility of these biomolecules, which retain their biological activity when bound, to microbial degradation. The persistence of bound insecticidal toxins may enhance the control of target pests, constitute a hazard to nontarget organisms, and result in the selection and enrichment of toxin-resistant target insects (Stotzky 2000).

Later the equilibrium adsorption and binding of the active toxin from *B. thuringiensis* subsp. kurstaki on complexes of montmorillonite-humic acids-Al hydroxy-polymers, as well as the biodegradation and the insecticidal activity of the bound toxin, were studied. 70% of the total adsorption occurred within the first hour, and maximal adsorption occurred in < 8 h. Adsorption of the toxin on a constant amount of the complexes increased as the amount of the toxin added increased, and equilibrium adsorption isotherms of the L-type were obtained. There was essentially no desorption of the toxin after extensive washing of the toxin-organomineral complexes with double distilled H_2O and 1 M NaCl. The bound toxin was resistant to utilization by mixed microbial cultures from soil and to enzymatic degradation by Pronase E. Free and bound toxins were active against the larvae of *Manduca sexta*; the bound toxin retained the same activity after exposure to microbes or Pronase, whereas the toxicity of the free toxin decreased significantly. It is obvious that the release of TPs and microorganisms expressing insecticidal Bt toxins could result in the accumulation of these toxins in soil as a consequence of binding on surface-active soil particles. It was stated again, that this persistence could pose a hazard to nontarget organisms, enhance the selection of toxin-resistant target species, and increase the control of target insect pests (Crecchio and Stotzky 2001). Equilibrium adsorption of the *B. thuringiensis* subsp. *israelensis* insecticidal toxins on the clay minerals montmorillonite and kaolinite, which are homoionic to various cations, was rapid (< 30 min for maximal adsorption), increased with protein concentration and then reached a plateau (68 to 96% of the proteins was adsorbed), was significantly lower on kaolinite than on montmorillonite, and was not significantly affected by the valence of the cation to which the clays were homoionic. Only 2 to 12% of the adsorbed proteins was desorbed by two washes with water; additional washings desorbed no more toxins, indicating that they were tightly bound. Formation of clay-toxin complexes did not alter the structure of the proteins. Free and clay-bound toxins resulted in 85 to 100% mortality of the mosquito *Culex pipiens* (Lee *et al* 2003). In a recent study it was shown that larvicidal proteins encoded by *cry* genes (Cry1Ab) were released in root exudates from transgenic corn, rice, and potato but not from Bt canola, Bt cotton, and Bt tobacco. Nonsterile soil and sterile hydroponic solution in which Bt corn, Bt rice, or Bt potato had been grown were immunologically positive for the presence of the Cry proteins; from Bt corn and Bt rice, the soil and solution were toxic to the larva of the tobacco hornworm (*Manduca sexta*), and from potato, to the larva of the CPB (*Leptinotarsa decemlineata*), representative lepidoptera and coleoptera, respectively. But no toxin was detected immunologically or by larvicidal assay in soil or hydroponic solution in which Bt canola, Bt cotton, or Bt tobacco, as well as all near-isogenic non-Bt plant counterparts or no plants, had been grown. The reasons for the differences between species in the exudation from roots of the toxins are not known. The released toxins persisted in soil as the result of their binding on surface-active particles (e.g. clay minerals, humic substances), which reduced their biodegradation. The authors suggested, that "the release of the toxins in root exudates could enhance the control of target insect pests, constitute a hazard to nontarget organisms, and/or increase the selection of toxin-resistant target insects (Saxena *et al* 2004).

(ii) *No*

Bt protected corn: In a recent laboratory study, Bt endotoxin in decomposing Bt corn and soil mixtures was rapidly degraded, with no detectable levels after only 14 days. It was suggested that much of the Bt endotoxin in crop residues is highly labile and quickly decomposes in soil, but that a small fraction may be protected from decay in relatively refractory residues (Hopkins and Gregorich 2003). In two field studies in the temperate corn-growing region of switzerland degradation of the crylAb toxin in transgenic Bt corn leaves during autumn, winter and spring using an enzyme-linked immunosorbent assay (ELISA) was investigated. In the first field trial, representing a tillage system, no degradation of the crylAb toxin was observed during the first month. During the second

month, crylAb toxin concentrations decreased to approximately 20% of their initial values. During winter, there was no further degradation. When temperatures again increased in spring, the toxin continued to degrade slowly, but could still be detected in June. In the second field trial, representing a no-tillage system, crylAb toxin concentrations decreased without initial delay as for soil-incorporated Bt plants, to 38% of the initial concentration during the first 40 days. They then continued to decrease until the end of the trial after 200 days in June, when 0·3% of the initial amount of crylAb toxin was detected (zwahlen *et al* 2003a,b).

Bt protected cotton: Soil samples were collected from within and outside six fields where Bt cotton (Bollgard) expressing (CrylAc) had been grown and subsequently incorporated into soil by postharvest tillage for 3–6 consecutive years. The level of CrylAc protein in these samples was evaluated using both ELISA and bioassays with a susceptible insect species, *Heliothis virescens* (F.), the tobacco budworm. Both methods revealed that no detectable CrylAc protein was present in any of the soil samples collected from within or outside the Bollgard fields. These findings demonstrated that the amount of CrylAc protein accumulated as a result of continuous use of Bt cotton, and subsequent incorporation of plant residues into the soil by postharvest tillage, is extremely low and does not result in detectable biological activity (Head *et al* 2002).

PI protected TPs: During the study of the transgenic tobacco plants producing PI I, it was demonstrated that under field conditions PI remained immunologically active in buried TP litter for at least 57 days and that decomposing parental and TPs litter differed in quality (carbon content) and in the response of exposed soil organisms (Collembola and nematodes) (Donegan *et al* 1997). The persistence of PI in buried TPs litter has been monitored immunologically. The concentration of PI in TPs litter had declined to 0·05% of the initial levels after 57 days in soil and could not be detected in subsequent samples (Donegan *et al* 1997).

3.1 *Will TPs adveresely affect soil organisms?*

One of the potential environmental effects of the recent rapid increase in the global agricultural area cultivated with transgenic crops is a change in soil microbially mediated processes and functions. Among the many essential functions of soil biota are soil organic matter decomposition, nutrient mineralization and immobilization, oxidation-reduction reactions, biological N fixation, and solubilization. The microbial communities exist within complex soil food webs together with numerous and varied soil-dwelling invertebrate species (e.g. earthworms, Collembola, and nematodes). Together, these communities carry out soil ecosystem processes such as nutrient cycling and decomposition, processes that have major ecological and agricultural significance. Soil-borne communities are dominated by microorganisms, which account for greater than 80% of the total biomass (excluding plant roots) (reviewed in Kowalchuk *et al* 2003; Dunfield and Germida 2004). There is the potential for soil-dwelling organisms to be exposed to transgene coded proteins released into the soil as exudates from the roots of living TPs (Borisjuk *et al* 1999) and through contact with plant material left in the ground after harvest. Moreover, there are special targeted genetic traits for improved plant nutrition which include greater plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic P, and increased assimilation of soil N. Among the potential direct effects of transgenic crops and their management are changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions resulting from gene transfer from the transgenic crop, and alteration in microbial populations because of the effects of management practices for transgenic crops, such as pesticide applications, tillage, and application of inorganic and organic fertilizer sources. Possible indirect effects of TPs, including changes in the fate of TPs residues and alterations in land use and rates of soil erosion, deserve further study. Despite widespread public concern, no conclusive evidence has yet been presented that currently released TP are causing significant direct effects on stimulating or suppressing soil nutrient transformations in field environments (reviewed in Motavalli *et al* 2004).

3.2 *Will TPs adveresely affect soil microorganisms?*

Early studies, in which the microbial populations associated with Bt plants were estimated by viable plating, failed to detect any impact that could be attributed to the genetic modification (Donegan *et al* 1995, 1996; Saxena and Stotzky 2001a). However, new molecular biology techniques, are beginning to provide insight into responses of soil microbial communities to TPs (Kowalchuk *et al* 2003; Bruinsma *el al* 2003).

(i) *Yes*

Bt protected TP: The rhizosphere bacterial community associated with Bt corn (Bt 176) and non Bt corn were characterized using several techniques, including viable counts, community-level catabolic profiling, and PCR-based fingerprinting, that targeted the 16S–23S intergenic transcribed spacers. The culturing techniques not detected any differences between the Bt corn and the non-

Bt plants, but the molecular profiling technique found that the community structure differed between the two treatments. Experiments in which root growth solutions were added to soil indicated that exudate of the Bt plant led to the development of a bacterial community that differed from that of the non-Bt plants. It was suggested that the Bt corn exudate may differ from the non-Bt plant in several ways, not only in the Cry protein (Brusetti *et al* 2004).

PI protected TPs: In the study on the effects of transgenic potato (expressing the PI cystatin) on nontarget organisms in the rhizosphere, phospholipid fatty acid analysis was used as a measure of the abundance, evenness, or metabolic activity of the soil microbial community. In the first year, two of the PI lines altered the structure of the community; one favoured fungal growth relative to bacterial growth in the later part of the season, and the second line appeared to suppress fungal growth. In the second year, phospholipid fatty acid analysis suggested that microbial abundance was reduced by 23%, with suppression of both bacterial and fungal communities. It is not known how cystatins from TPs affect microorganisms. Free cystatin is likely to be rapidly inactivated by enzyme activity or by adsorption to solid surfaces, and the observed changes in the microbial community may have reflected changes in the composition of the root exudates of the TPs and not inhibition of microbial proteinase activity (Cowgill *et al* 2002).

Lectin protected TPs: Community-level physiological profiling of soil surrounding lectin-expressing potato roots found that, although transgenic potato lines consistently altered the physiological profile of the rhizosphere microbial community at harvest, the effect did not persist from one season to the next over a trial period of two field seasons (Griffiths *et al* 2000).

HT transgenic plants: The soybean nitrogen fixing symbiont, *Bradyrhizobium japonicum*, possesses a glyphosate-sensitive enzyme and upon exposure to glyphosate accumulates shikimic acid and hydroxybenzoic acids such as protocatechuic acid (PCA), accompanied with *B. japonicum* growth inhibition and death at high concentrations. In a series of greenhouse and field experiments, glyphosate inhibited nodulation, and nodule leghaemoglobin content of HT soybean. Glyphosate accumulated in nodules of field-grown HT soybean, but its effect on nitrogenase activity of HT soybean was inconsistent in field studies. In greenhouse studies, nitrogenase activity of HT soybean following glyphosate application was transiently inhibited especially in early growth stages, with the greatest inhibition occurring under moisture stress. Studies using bacteroid preparations showed that the level of glyphosate inhibition of bacteroid nitrogenase activity was related to *in vitro* glyphosate sensitivity of the *B. japonicum* strains. These studies indicated the potential for reduced nitrogen fixation in the HT soybean system; however, yield reductions due to this reduced N2 fixation in early stages of growth have not been demonstrated (Zablotowicz and Reddy 2004).

Other TPs: A field study using transgenic alfaalfa with associated recombinant microorganisms was conducted to assess their potential effects on soil ecosystems. Three lines of alfalfa plants (parental, transgenic alpha-amylase-producing and transgenic lignin peroxidase-producing) were planted in an agricultural field plot. Immediately prior to planting, the roots of the alfalfa plants were left uninoculated or were inoculated with a wild-type strain (PC), a recombinant strain with antibiotic resistances (RMB7201), or a recombinant strain with antibiotic resistances and enhanced nitrogen-fixation capability (RMBPC-2), of *Sinorhizobium meliloti*. Analyses of the alfalfa plants and field plot soil were made over two growing seasons and included: metabolic fingerprints and DNA fingerprints of soil bacterial communities; soil microbial respiration; population counts of indigenous soil bacteria, fungi, nematodes, protozoa and micro-arthropods; identification of nematodes and micro-arthropods; plant shoot weight and chemistries; and soil chemistries and enzyme activities. The lignin peroxidase transgenic plants had significantly lower shoot weight, and higher nitrogen and phosphorus content, than the parental or transgenic amylase plants. Distinct metabolic fingerprints, based on patterns of substrate utilization in Biolog plates, were exhibited by the soil bacterial communities associated with the three alfalfa genotypes, and those for the lignin peroxidase plants were the most unique. Significantly higher population levels of culturable, aerobic spore-forming and cellulose-utilizing bacteria, lower activity of the soil enzymes dehydrogenase and alkaline phosphatase, and higher soil pH levels, were also associated with the lignin peroxidase transgenic plants. Significantly higher population levels of culturable, aerobic spore-forming bacteria were also measured in the treatments containing the recombinant RMBPC-2 *S. meliloti*.

Population levels of protozoa, nematodes and micro-arthropods, DNA fingerprints of indigenous soil bacteria, and rates of microbial substrate-induced respiration were not significantly affected by the transgenic alfalfa and recombinant *S. meliloti* treatments.

Authors claimed, that “the transgenic organisms caused detectable changes in some components of the soil ecosystem. The primary effects wich was observed were associated with the transgenic lignin peroxidase alfalfa and included alterations in plant growth and chemistry and changes in soil chemistry and microbiology” (Donegan *et al* 1999).

(ii) *No*

Bt protected TPs: Bt potato plants was monitored for changes in total bacterial and fungal populations, fungal species diversity and abundance, and plant pathogen levels. The microflora on three phenological stages of leaves were compared over the growing season for (i) transgenic potato; (ii) plants, commercial Russet Burbank; (iii) potato plants treated with systemic insecticide (Di-Syston) and commercial; and (iv) Russet Burbank potato plants treated with microbial Btt (M-Trak). In addition, plant and soil assays were performed to assess disease incidence of *Fusarium* spp., *Pythium* spp., *Verticillium dahliae*, potato leaf roll virus (PLRV) and potato virus Y (PVY). Few significant differences in phylloplane microflora among the plant types were observed and none of the differences were persistent. It was concluded that under field conditions the microflora of transgenic Bt potato differed minimally from that of chemically and microbially treated commercial potato plants (Donegan *et al* 1996).

PI protected TPs: Plant-parasitic nematodes are important pests of agriculture and TPs with potential for nematode control are currently being developed. The expression of cysteine proteinase inhibitors (cystatins) in transgenic potato confers partial resistance to PCN. The field studies was used to test effects of the transgenic potato on non-target soil organisms. Microbial community structure, soil microarthropods and litter decomposition were studied during two growing seasons. In the second year, nematode control options of cystatin-expressing plants and an oxime carbamate nematicide application were compared for their non-target effects. In the first year, the transgenic lines had no effect on the abundance, evenness or metabolic activity of the soil microbial community as determined by ester-linked phospholipid fatty acid analysis (PLFA). However, one transgenic line (D6/7) influenced the structure of the soil microbial community. PLFA suggested it favoured fungal growth relative to bacterial growth during the latter parts of the growing season. A second transgenic line (D5/13) was more effective against PCN. It reduced the abundance of the fungal fatty acid 18 : 2 omega 6 in late season, suggesting a suppression of fungal growth. In the second year PLFA analysis suggested microbial abundance was reduced by 15% and 23% in the nematicide and transgenic treatments, respectively, relative to the control. Nematicidal treatment reduced the bacterial fraction of the microbial community, whereas the transgenic plants suppressed both the bacterial and fungal community components. The observed changes in soil microbial community structure did not resulted in changes in the rate of leaf litter decomposition. The transgenic lines had no significant effect on the abundance of soil microarthropods or free-living nematodes. Authors suggested that the study is the first stage of a risk assessment of the impact of transgenic nematode resistance on non-target soil organisms. Both nematicide and the transgenic plants affected components of the soil microbial community. However, the changes brought about by the two treatments were not sufficient to affect soil functioning, as measured by rates of litter decomposition (Cowgill *et al* 2002b).

T4-lysozyme protected TPs: Transgenic potato that produce bacteriophage T4-lysozyme for enhanced bacterial resistance was monitored for changes in plant-associated bacterial populations, in the functions of potentially beneficial bacteria and in the diversity of antagonistic bacterial species. These parameters have been analysed for transgenic and non-transgenic lines, over a period of 2 years at different stages of plant development and at two different locations. Two microenvironments, the rhizo- and geocaulosphere, were investigated. No significant differences in aerobic plate counts were observed between the four plant lines. In addition, no significant differences in the functions of potentially beneficial and antagonistic bacteria (antagonists to *Erwinia carotovora* and *Verticillium dahliae*) were found. The diversity of antagonistic species isolated from each plant line and microenvironment was investigated to determine if the diversity and composition of potentially beneficial bacteria were influenced. Altogether, 28 different potato-associated species with antagonistic effects to phytopathogens were detected. Antagonistic strains of seven species were found only on control plants. The observed effect was minor relative to the natural variability observed during the monitoring period plants under a variety of environmental conditions (Lottmann *et al* 1999).

Chitinase protected TPs: Another risk evaluation was performed with a transgenic strawberry expressing a rice chitinase gene. The study was carried out in a closed greenhouse, a semi-closed greenhouse, and an isolated field. The transgenic strawberry not produced any specific products, for example compounds secreted from the roots or volatile compounds released from the plant. The influence of transgenic strawberry cultivation on the growth of other plants and soil microflora was investigated in a semi-closed greenhouse. It was concluded that the effect of the transgenic strawberry on other plants (radish and spinach) and microflora (fungi, bacteria, and actinomycetes) was no different from that of a non-transgenic strawberry. Furthermore, there was no difference between the transgenic and the non-transgenic strawberries in terms of morphological characteristics and yield except for disease resistance (Asao *et al* 2003).

HT transgenic plants: Bacterial communities in rhizospheres of transgenic corn (*Zea mays*, with the pat-gene

conferring resistance to the herbicide glufosinate; syn. L-phosphinothricin) were compared to its isogenic, non-transgenic cultivar. With the usage of single-strand conformation polymorphism (SSCP) it was found that genetic profiles of rhizospheres consisted of 40–60 distinguishable bands depending on the chosen polymerase chain reaction (PCR) primer pairs, and the variability between independent replicates was very low. Neither the genetic modification nor the use of the herbicide liberty (syn. Basta active ingredient: glufosinate) affected the SSCP. In contrast, PCR-SSCP profiles of bacterial communities from rhizospheres of sugar beet, grown in the same field as a control crop, were clearly different. A less pronounced but significant difference was also observed with rhizosphere samples from fine roots of corn plants collected 35 and 70 days after sowing. Sequencing of the dominant 30 products from one typical SSCP profile generated from transgenic corn rhizospheres indicated the presence of typical soil and rhizosphere bacteria: half of the bands could be attributed to Proteobacteria, mainly of the alpha- and beta-subgroups. Other SSCP bands could be assigned to members of the following phylogenetic groups: Cytophaga-Flavobacterium-Bacteroides, Chlamydiales-errucomicrobium, Planctomyces, Holophaga and to Gram-positive bacteria with a high G+C DNA content (Schmalenberger and Tebbe 2002).

In the field study, the bacterial communities inhabiting the rhizosphere of a transgenic, herbicide-resistant sugar beet (*Beta vulgaris*) cultivar was compared with those of its non TP using PCR and SSCP. As a control for the plasticity of the bacterial community, it was also analysed the influence of herbicides, the field heterogeneity, and the annual variation. DNA was isolated from bacterial cell consortia that were directly collected from root material. Patterns of the replicates and the different treatments were highly similar, but digital image and similarity analyses revealed differences that corresponded to the positions of the replicates in the field. In addition, communities collected from sugar beet in two successive growing seasons could be distinguished. In contrast, no effect of the transgenic herbicide resistance was detectable. Sequencing of 24 dominant products of the SSCP profiles indicated the presence of bacteria from different phylogenetic groups, with Proteobacteria and members of the Cytophaga-Flavobacterium-Bacteroides group being most abundant (Schmalenberger and Tebbe 2003).

A two year, multiple-site field study was condacted in which rhizosphere samples associated with a HT transgenic canola variety and a conventional canola variety were sampled at six times throughout the growing season. The objectives of this study were to identify differences between the rhizosphere microbial community associated with the transgenic plants and the rhizosphere microbial community associated with the conventional canola plants and to determine whether the differences were permanent or depended on the presence of the plant. Community-level physiological profiles, fatty acid methyl ester profiles, and terminal amplified ribosomal DNA restriction analysis profiles of rhizosphere microbial communities were compared to the profiles of the microbial community associated with an unplanted, fallow field plot. Principal-component analysis showed that there was variation in the microbial community associated with both canola variety and growth season. Importantly, while differences between the microbial communeties associated with the transgenic plant variety were observed at several times throughout the growing season, all analyses indicated that when the microbial communities were assessed after winter, there were no differences between microbial communities from field plots that contained harvested transgenic canola plants and microbial communities from field plots that did not contain plants during the field season. Hence, the changes in the microbial community structure associated with genetically modified plants were temporary and not persisted into the next field season (Dunfield and Germida 2003).

3.3 *Will TPs adveresly affect arbuscular mycorrhizal fungi?*

(i) *Yes*

TPs that constitutively express proteins with potential antifungal and/or antibacterial activity, can reduce activities of specific soil-borne plant pathogens in the rhizosphere (reviewed in Glandorf *et al* 1997). Arbuscular mycorrhizal (AM) fungi establish mutualistic symbioses with roots of most plant species, playing an important role in soil fertility and plant nutrition. AM fungi are strongly affected by agricultural practices and changes in soil characteristics and thus are key nontarget microorganisms worthy of monitoring in environmental impact assessments of TPs (Bruinsma *et al* 2003). The expression of the chitinase is believed to increase the transgenic host's nonspecific basic resistance to pathogens. A potential nontarget effect of constitutively expressing chitinase may be a decrease in the activity of vesicular-arbuscular mycorrhizal fungi. The decrease in activity of mycorrhizal fungi was related to reduced susceptibility of TPs plant roots to colonization by these fungi, which is in turn associated with lysis of fungal cell walls by the constitutively expressed chitinase. Early data demonstrated a strong negative association between transgenic host pathogen resistance and mycorrhizal colonization. An ecological consequence of reducing mycorrhizal colonization was a decrease in the soil's mycorrhizal propagule reserve that diminishes the next crop's production, especially under low-input cropping practices (Miller 1993). Constitutive

expression of antifungal pathogenesis-related proteins in tobacco in most cases not affected root colonization by the mycorrhizal fungus *Glomus mosseae*. However, increased levels of a class II tobacco beta-1,3-glucanase reduced the colonization potential, indicating that nontarget effects can occur (Glandorf *et al* 1997). Root exudates of one line of Bt corn (event 176) significantly reduced presymbiotic hyphal growth of *G. mosseae* compared with another line (event-11) and control plant root exudates. Development of appressoria was also affected by Bt event 176, with 36% of appressoria failing to produce viable infection pegs. Event 176 had a higher level of expression of the Cry1Ab toxin than did event-11 (Turrini *et al* 2004).

In general, the potential for interaction between TPs and plant residues and the soil microbial community is not well understood. Furthermore, transgenic proteins have been shown to be released from transgenic plants into the soil ecosystem, and their presence can influence the biodiversity of the microbial community by selectively stimulating the growth of organisms that can use them. Microbial diversity can be altered when associated with TPs; however, these effects are both variable and transient. Soil- and plant-associated microbial communities were influenced not only by plant species and transgene insertion but also by environmental factors such as field site and sampling date (reviewed in Dunfield and Germida 2004; Motavalli *et al* 2004).

3.4 *Will transgenic plants adversely affect soil microarthropods?*

Collembolans are recognized as the key indicator species of soil fertility and health, as they play a vital role in the break down and recycling of crop residues and abundant populations of these microarthropods are generally present in well-managed agricultural soils. Collembolans are often found in the root zone of plants and can be exposed to transgene-derived proteins exuded into the rhizosphere by roots. As collembolans are active in the decomposition of organic matter, they may also be exposed to transgene-derived proteins that remain in crop residues.

(i) *Yes*

PI protected TPs: When tobacco leaves expressing P II were buried in the field, there were significantly lower collembolan numbers in the surrounding soil than in soil from control plots. The high mobility of Collembola made it difficult to determine whether the depressed numbers in soil surrounding the TPs litterbags were due to mortality and/or decreased reproduction or merely to increased migration (Donegan *et al* 1997).

(ii) *No*

Bt protected TPs: The effects of Bt endotoxins [HD-1 CryIA(b) and HD-73 CryIA(c)] producing in transgenic cotton and potato on 2 nontarget soil arthropods, a collembolan, *Folsomia candida* Willem, and an orbatid mite, *Oppia nitens* Koch were tested. It was found that time to oviposition, egg production, and final body length were unaffected when *F. candida* were fed residues of Bt cotton. Total production of *O. nitens* adults and nymphs was unaffected by feeding on leaves of Bt cotton and Bt potato (Yu *et al* 1997). Similarly, purified Bt toxins and leaf tissue of Bt cotton, Bt corn, and Bt potatoes had no effect on *F. candida* and *Xenylla grisea* (United States Environmental Protection Agency 2000). Also there was no effect of maize expressing the coleopteran-active Bt toxin Cry3Bb1 on field populations of Collembola and soil mites over two seasons (Al-Deeb *et al* 2003).

PI protected TPs: Trangenic potatoes expressing cysteine PI had no effect on the abundance of collembolans during two field seasons (Cowgill *et al* 2002a). There was no effect on the abundance of mites in field soil collected from around the roots of potatoes expressing PIs (Cowgill and Atkinson 2003).

Other TPs: Two different transgenic wheat varieties expressed a gene from an *Ustilago maydis*-infecting virus. The gene product (KP4 protein) is known for its growth inhibitory activity against fungi in the *Ustilaginales*. These KP4-transgenic wheat plants showed an enhanced resistance against stinking smut, *Tilletia tritici*. Different laboratory bioassays and a glasshouse study where with feeding bioassays in which dried root material from transgenic and non-transgenic wheat plants was fed to individual *candida* revealed no effect of transgenic wheat variety on any of the life-history parameters evaluated. The glasshouse study showed that population development of *F. candida* not differed between transgenic or non-transgenic plants of the two varieties were grown. However, a significant variety effect was detected (Romeis *et al* 2003b).

3.5 *Will TPs adversely affect earthworms?*

Earthworms play a vital role in decomposition of plant litter, and the physical and chemical changes to soil resulting from earthworm activity can significantly affect on the soil's biological properties and processes. Earthworms could ingest the bound toxins and, may be could be affected by them. Moreover, earthworms may function as intermediaries through which the toxins are passed on to other trophic levels. Are there an evidence of a negative effects of TPs on soils earthworms have been found?

(i) *Yes*

It was found that adult earthworms *Lumbricus terrestris* L. lost 18% of their initial weight after 200 days when fed Bt corn compared with a 4% weight gain when fed non-Bt corn. The authors were uncertain whether this effect was caused by the Bt toxin and suggested that it may have resulted from a difference in the nutritional quality of the plant material ingested by the earthworms (Zwahlen *et al* 2003a,b).

(ii) *No*

Tests on the effects of the purified Bt toxin on earthworms suggested that Bt plants have little impact on earthworms. For example, there were no growth effects or mortality in *Eisenia foetida* exposed to 120 times the amount of Cry3A toxin expected in one kilogram of soil in a plot of Bt potatoes (United States Environmental Protection Agency 2000). It was shown that earthworms can ingest Bt toxin bound to soil. In this study, Cry1Ab was detected in the guts and casts of earthworms kept for 45 days in jars containing Bt maize biomass. No deleterious effects of Bt maize on *L. terrestris*) were detected in these pot trials (Saxena and Stotzky 2001a).

3.6 *Will TPs adversely affect soil nematodes?*

Soil nematodes are a diverse group of organisms that are found in all soils. Nematodes that feed on rhizosphere bacteria, fungi, protozoa, rotifers, or other nematodes may conceivably be affected by the presence of TPs litter or root exudates in the soil.

(i) *Yes*

Lectin protected TPs: In laboratory experiments carried out as part of a study on the effects of trangenic potatoes expressing lectins (concanavalin A and GNA), the host-finding response of a bacteria-feeding nematode was significantly inhibited when either lectin was present at a range of concentrations (Griffiths *et al* 2000).

PI protected TPs: Litterbags containing leaves of parental and transgenic tobacco plants producing proteinase inhibitor I were buried in field plots. The litterbag contents and surrounding soil, as well as soil from control plots without litterbags, were sampled over a 5-month period at 2- to 4-week intervals and assayed for proteinase inhibitor concentration, litter decomposition rates, carbon and nitrogen content, microbial respiration rates and population levels of nematodes, protozoa and microarthropods. The proteinase inhibitor concentration in the TPs litter after 57 days was 0·05% of the sample day 0-value and was not detectable on subsequent sample days. Although the carbon content of the TPs litter was comparable to that of the parental plant litter on sample day 0, it became significantly lower over the course of the experiment. Nematode populations in the soil surrounding the TPs litterbags were greater than those in the soil surrounding parental plant litterbags and had a different trophic group composition. In contrast, Collembola populations in the soil surrounding the TPs litterbags were significantly lower than in the soil surrounding parental plant litterbags. It was concluded, that under field conditions proteinase inhibitor remained immunologically active in buried TPs litter for at least 57 days and that decomposing parental and TPs litter differed in quality (carbon content) and in the response of exposed soil organisms (Collembola and nematodes) (Donegan *et al* 1997).

(ii) *No*

Bt protected TP: There were no differences in nematode populations in soil close to the roots of maize expressing the Bt toxin Cry3Bb1 and of non-Bt plants (Al-Deeb *et al* 2003).

PI protected TPs: In pot trials and decomposition experiments carried out in the same study, there was no effect on nematode populations. After two field seasons, there were no differences in the abundance of free-living nematodes around the roots of PI protected potato plants expressing cysteine PIs (cystatins), even though the target of this modification was the potato cyst nematode (Cowgill and Atkinson 2003).

3.7 *Will TPs adversely affect isopods?*

(i) *No*

No adverse effects of Bt corn (Cry1Ab) were found on woodlice *Porcellio scaber* in a soil-free laboratory system.Woodlice did not differentiated between Bt corn and non Bt corn in their food preference, and numbers of offspring produced did not differ between the two treatments. Initial weight increases of offspring were significantly higher in the non transgenic treatment, but adult *P. scaber* showed greater weight increase when feeding on Bt corn leaves. Differential weight gains between the treatments may have resulted from differences in the nutritional quality of the Bt and non-Bt corn leaves (Escher *et al* 2000).

3.7 *Wiil TPs adveresly affect Protozoa?*

(i) *Yes*

Lectin protected TPs: Protozoa are key components of the soil food web and play an important role in soil nutri-

ent cycling. In a pot experiment, flagellate protozoan populations in the rhizosphere of one transgenic potato expressing the lectin concanavalin A were significantly lower than that of control plant and GNA plant rhizosphere. In addition, significantly fewer protozoa and amoebae were found in the presence of leaves from lectin-expressing transgenic lines than in the control lines. When a single concanavalin A-producing line was grown in the field, there was a transient reduction of about 40% in soil protozoan population (Griffiths *et al* 2000).

3.8 *Will TPs adverelsy affect soil processes?*

Only limited number of studies have measured effects of TPs on soil processes; plant litter decomposition has most often been chosen as a key indicator of soil ecosystem function.

(i) *Yes*

It was reported that biomass of Bt corn decomposed less in soil than biomass of near-isogenic, non-Bt corn (Stotzky 2002). During the study of ecological impacts of Bt corn (Cry1Ab) fluorescence microscopy and staining with toluidine blue indicated a higher content of lignin in the vascular bundle sheaths and in the sclerenchyma cells surrounding the vascular bundle in all ten Bt corn hybrids, representing three different transformation events, studied than of their respective non-Bt isolines. Chemical analysis confirmed that the lignin content of all hybrids of *Bt* corn, whether grown in a plant growth room or in the field, was significantly higher (33–97% higher) than that of their respective non-*Bt* isolines. As lignin is a major structural component of plant cells, modifications in lignin content could have ecological implications. For example, the increase in lignin content in *Bt* corn may be beneficial, as it can provide greater resistance to attack by second-generation European corn borer (ECB) reduce susceptibility to molds and retard litter degradation and decomposition by microbes. The addition of biomass from *Bt* corn to soil resulted in a significantly lower gross metabolic activity (i.e. CO_2 evolution) in soil than did the addition of non-*Bt* corn, which may be beneficial, as the organic matter derived from *Bt* corn may persist and accumulate longer and at higher levels in soil, thereby improving soil structure and reducing erosion or it may be detrimental, as the longer persistence of the biomass of *Bt* corn may extend the time that the toxin is present in soil and, thereby, may enhance the hazard to nontarget organisms and result in the selection and enrichment of toxin-resistant target insects. Moreover, lignin is relatively indigestible and reduces the ability of herbivores to digest plant material, and its increase in forages might affect rates of feeding and population dynamics of defoliators (Saxena and Stotzky 2001a,b).

Indeed, it seems, that soil respiration could be negatively affected by TPs. Respiration was measured in soil samples to which Bt corn Cry1Ab and non-Bt corn shoots had been added and in soils collected when field-grown crops were harvested. It was found that, Bt corn appeared to decompose more slowly than non-Bt corn and the cumulative CO_2–C evolved from soils was significantly lower under Bt crops than under non-Bt crops. However, this study contained anomalous results; the net rate of CO_2 production increased throughout the duration of the experiment, whereas this rate usually declines over time as less organic matter is available in soil. Bt corn and non-trangenic shoots were reported to differ in the amount and type of fatty acids they contained, as did soils from beneath field crops. The authors suggested that the cultivation of Bt corn significantly increased the saturated-to-unsaturated lipid ratios in soils, which appeared to affect microbial activity negatively. However, further investigation is required before this conclusion can be drawn (Dinel *et al* 2003).

The impacts of the amendment of Bt-transgenic rice *Cry1Ab* (KMD) straw on biological activities in water-flooded soil were investigated under laboratory conditions and compared with non-transgenic rice (Xiushui 11) straw. The results showed that there were some differences in protease, neutral phosphatase and cellulase activities between soil amended with Bt-transgenic rice straw and non-transgenic rice straw at the early stage of incubation, and none of these differences were persistent. However, differences in dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration between soil supplemented with Bt-transgenic rice straw and non-transgenic rice straw were persistent over the course of incubation. Dehydrogenase activity, methanogenesis and anaerobic respiration were considerably lower from sample days 7 to 56, but higher after day 56 in soil amended with Bt-transgenic rice straw. In comparison, the H_2-production in soil containing Bt-transgenic rice straw was significantly lower after day 56. The results demonstrated that the amendment of the Bt-transgenic rice straw altered some important biological properties in water-flooded soil, indicating a shift in microbial populations or a change in the metabolic abilities of the microbial community as a result of substrate availability in soil (Wei-Xiang Wu *et al* 2004).

(ii) *No*

On the other hand several studies have found little difference between the decomposition rates of non TPs and TPs. Rate of decomposition of potato (Cowgill *et al* 2002a,b) and tobacco leaves (Donegan *et al* 1997) from plants modified to express a PI were similar to those of comparative non-TPs as measured by weight loss in lit-

terbag studies. Similarly, no detectable difference between the decomposition rates of Bt corn and non-Bt corn, as determined by CO_2 production, was found (Hopkins and Gregorich 2003).

4. Will introgression occur?

What are the possible ways of introgression and what is the probability that this "worst case scenario" will proceed? Introgression could occur by transgenic pollen which will fertilize non transgenic plants and by microorganisms which will capture transgenes and hitch-hike them into the world wide web of genes exchange.

4.1 *Will trangenic DNA persist in a soil for a long time?*

According to analyses of complete genome sequences horizontal gene transfers have played a fundamental role in bacterial evolution. Nowadays, this efficiency of bacteria in picking up genes of surrounding organisms that rises concerns about a potential dissemination of genes from TPs to soil bacteria. Theoretically, it is not excluded, that transgenes, released from TPs into the soil could be captured by microorganisms and then transfered into other organisms. Is this type of the risk considerable? The sets of the special studies were aimed to study the different stages of such undesirable scenario. What will be the potential fate and effects of transgenes released form TPs into the soil, for example form TPs engineered to produce vaccines, hormones, antibodies, toxins, pharmaceuticals and containing the correspondiding genes?. Will these transgenes coding such physiologically active proteins 'survive' in a soil in a potentially transforming state?

(i) *Yes*

As a model system for studying the persistense of transgenes, the stability of cloned neomycin phosphotransferase II (rNPT-II), a neomycin/kanam in the soilsycin resistance marker was tested. The recombinant nature of the target (i.e. fusion of nopaline synthase promoter and NPT-II coding region) allowed to design a rNPT-II-specific PCR primer pair. Effects of temperature and moisture, on DNA persistence in soil were determined in two laboratory test systems. In the first system, purified plasmid DNA was added to soil and incubated under controlled conditions. Up to 0·08% of the rNPT-II target sequences were detectable after 40 days. In the second system, fresh leaf tissue of transgenic tobacco was ground, added to soil, and incubated under controlled conditions. After 120 days, up to 0·14% of leaf tissue-derived genomic rNPT-II sequences were detectable. Under most experimental conditions, leaf tissue-derived and plasmid DNA were initially degraded at a high rate. A small proportion of the added DNA resisted degradation and was detectable for several months. It was proposed that this DNA may have been adsorbed to soil particles and was protected from complete degradation (Widmer *et al* 1996; Stotzky 2000).

4.2 *Can transgenic plants DNA transform indigenous microbes?*

The only mechanism which could be involved for such gene transfer is natural transformation which requires the successive occurrence of several steps, including release of plant DNA, persistence of DNA in the environment presence of transformable bacteria and internalization of the sequences in the new host. So, can transgenes transform soil microorganisms in fact?

(i) *Yes*

It was shown that horizontal transfer of DNA, extracted from transgenic sugar beets, to bacteria, based on homologous recombination, can occur in soil. Restoration of a 317-bp-deleted *nptII* gene in *Acinetobacter* sp. strain BD413(pFG4) cells incubated in sterile soil microcosms was detected after addition of nutrients and transgenic plant DNA encoding a functional *nptII* gene conferring bacterial kanamycin resistance. Selective effects of the addition of kanamycin on the population dynamics of *Acinetobacter* sp. cells in soil were found, and high concentrations of kanamycin reduced the colony forming unit (CFU) of *Acinetobacter* sp. cells from 10^9 CFU/g of soil to below detection. In contrast to a chromosomal *nptII*-encoded kanamycin resistance, the pFG4-generated resistance was found to be unstable over a 31-day incubation period *in vitro* (Nielsen *et al* 2000). Transgenic potato plants with the *nptII* gene coding for neomycin phosphotransferase (kanamycin resistance) as a selection marker were examined for the spread of recombinant DNA into the environment. The recombinant fusion of *nptII* with the tg4 terminator was used for a novel biomonitoring technique. This depended on natural transformation of *Acinetobacter* sp. strain BD413 cells having in their genomes a terminally truncated *nptII* gene (*nptII* kanamycin sensitivity) followed by the tg4 terminator. Integration of the recombinant fusion DNA by homologous recombination in *nptII* and tg4 restored *nptII*, leading to kanamycin-resistant transformants. DNA of the transgenic potato was detectable with high sensitivity, while no transformants were obtained with the DNA of other transgenic plants harbouring *nptII* in different ge-

netic contexts. The recombinant DNA was frequently found in rhizosphere extracts of transgenic potato plants from field plots. In a series of field plot and greenhouse experiments two sources of this DNA spread by roots during plant growth and by pollen during flowering was identified. Both sources also contributed to the spread of the transgene into the rhizospheres of nontransgenic plants in the vicinity. The longest persistence of transforming DNA in field soil was observed with soil from a potato field in 1997 sampled in the following year in April and then stored moist at 4°C in the dark for 4 years prior to extract preparation and transformation (de Vries *et al* 2003). PCR monitoring of field releases of transgenic sugar beet plants demonstrated that during the growth of the plants the soil close to the plants and also plant material contains recombinant DNA, in the form of extracellular molecules. Surprisingly, the monitoring also revealed the presence of transgenic DNA in many field plots (30–70%) in which TPs plants were never grown. These studies and the further monitoring during other transgenic sugar beet release experiments by PCR and a novel bioassay (measuring the transforming potential of recombinant DNA for *Pseudomonas stutzeri*) indicated that recombinant DNA was only detectable in the surface soil of field plots and their vicinity where flowering of the transgenic beet plants was allowed. Transgenic DNA was found in soil at a distance of 50 m from pollen-producing plants surrounded by a strip with hemp plants as a containment regime. It was concluded that transgenes was deposited in soil during the growth of transgenic plants and that a major mechanism of transgens spread in the environment is the dispersal of pollen which allows transgenes to persist in the field plot for at least a year (Meier and Wackernagel 2003).

In general, the available data indicated that transformation-mediated gene transfers could occur in the environment and in the case of transgenic plants are more likely to involve the prokaryotic sequences of the transgene than the remaining of the genome (reviewed in Nielsen *et al* 2001). Indeed, in the recent study DNA transfer was demonstrated from six species of donor plants to the soil bacterium, *Acinetobacter* spp. BD413, using neomycin phosphotransferase (*nptII*) as a marker for homologous recombination. These laboratory results were compatible with, but do not prove, DNA transfer in nature. In tobacco carrying a plastid insertion of *nptII*, transfer was detected with 0·1 g of disrupted leaves and in oilseed rape carrying a nuclear insertion with a similar quantity of roots. Transfer from disrupted leaves occurred in sterile soil and water, without the addition of nutrients. It was detected using intact tobacco leaves and intact tobacco and *Arabidopsis* plants *in vitro*. Transfer was dose dependent and sensitive to DNase, and mutations in the plant *nptII* were recovered in receptor bacteria. DNA transfer using intact roots and plants *in vitro* was easily demonstrated, but with greater variability. Transfer varied with plant genome size and the number of repeats of the marker DNA in the donor plant. Transfer was not detected in the absence of a homologous *nptII* in the receptor bacteria (Tepfer *et al* 2003). However, soil bacteria use to exchange genes by conjugation at frequencies several orders of magnitude higher than those resulting from a transfer from plants. One can thus assess that ecological consequences of interkingdom transfers of plant genes, naturally present in soil bacteria and which do not modify the fitness of the recipient microorganism would remain negligible (Simonet 2000).

4.3 *Will trangenic pollen dispers onto non transgenic fields?*

The most serious concerns have been raised about the potential effects of transgenic introductions on the genetic diversity of crop landraces and wild relatives in areas of crop origin and diversification, as this diversity is considered essential for global food security. The Nuffield Council on Bioethics suggests that introgression of genetic material into related species in centres of crop biodiversity is an insufficient justification to bar the use of genetically modified crops in the developing world. They consider that a precautionary approach to forgo the possible benefits invokes the fallacy of thinking that doing nothing is itself without risk to the poor (Nuffield Bioethics Committee 2004). Pollen dispersal has been recently focused on as a major issue in the risk assessment of TPs. The possible risks posed by cross hybridization with wild relatives have been extensively explored (reviewed in Stewart *et al* 2003).

(i) *Yes*

In fact, the shape of the pollen dispersal of individual plants is hard to determine since a very large number of plants must be monitored in order to track rare long-distance dispersal events. Conversely, studies using large plots as a pollen source provide a pollen distribution that depends on the shape of the source plot. Border rows have been used frequently to restrict the pollen-mediated escape of transgenes from held trials, but what are the real efficacy of this approach? To test this, isogenic lines of cucumber (*Cucumis sativus* L.) differing for the seedling marker trait blunt leaf apex (bla) were planted in four designs: 1m^2 of wild-type donor plot surrounded by a 399 m^2 border of bla recipients; 1 m^2 of donor plot/99 m^2 border; 4 m^2 of donor plot/96 m^2 border; and 1 m^2 of donor plot with no border. Each planting was encircled by eight 1·4 m^2 satellite plots 50 m from the plot center.

Progeny of plants from the satellites and borders were screened to determine the percentage of gene movement as measured by the occurrence of the dominant phenotype. Gene movement within the plot borders followed a leptokurtic distribution; there was greater movement from the 4 m^2 donor plot than from the 1 m^2 plot. Long-distance movement to the satellites significantly increased as the trap-to-donor ratio decreased. Although movement to individual satellites was generally consistent within a treatment, there was one instance of unusually high out crossing to a single satellite, indicating that the effectiveness of borders was influenced by both the relative numbers of donor plants and by environmental variables (Hokanson *et al* 1997).

4.4 *Will transgenic pollen provide hybrization between transgenic and non transgenic plants?*

The potential for ecological and agricultural risks through introgression is based on the occurrence of transgenic hybrid populations. The logical step in assessing these risks is to determine the frequency with which these hybridizations occur. The introgression of transgenes into a wild species or population occurs in a step-wise fashion starting with the initial hybridization between the transgenic crop and the wild relative. The frequency of hybridization may be affected by many factors, including the transgene insertion locus within the plant, the agronomic properties of the transgenic variety, the breeding system of the wild relative, the relative compatibility of the crop and weed species, the crop to wild relative ratio, the distance between the transgenic crop and wild relative, and the environmental conditions in the field (Stewart *et al* 2003).

(i) *Yes*

The introgression of transgenic DNA in crop landraces is of critical importance. The first report about the presence of transgenic DNA in landraces (of native corn or *criollo* in Mexico's Oaxaca province) appeared in 2001. Oaxaca lies within the centre of origin and diversification of corn, which extends from Peru to Mesoamerica. The samples were collected from four fields of *criollo* 20 km from the highway that crosses the remote Sierra Norte de Oaxaca, together with bulk seed from Diconsa, the local Mexican government seed distribution agency. The control samples was from a 1971 corn collection from Oaxaca, and cobs from the Cuzco Valley of Peru; together with two transgenic corn cultivars, one Bt-protected, the other resistant to commercial 'Roundup' herbicide. PCR revealed substantial transgenic contamination of the Diconsa sample by regulatory DNAs used in plant genetic engineering: 35S promoter from the cauliflower mosaic virus (CMV) and T-NOS terminator of nopaline synthase sequences form *Agrobacterium tumefaciencs*. This compared with transgenic levels found in the control transgenic corn cultivars. The four field samples showed smaller but significant amounts of transgenic DNA. There was, however, no evidence for transgenes in the Peruvian sample or the 30-year old Oaxaca sample (Quist and Chapela 2001). In spite of the followed criticism (Metz and Futterer 2002; Kaplinsky *et al* 2002) a concurrent independent study by Mexican scientists has also confirmed transgenic DNA in land-races in Oaxaca, and elsewhere in the country (Pearce 2002), The diversity of the transgenic DNA, apparently presented on chromosomes at multiple loci, suggested repeat episodes of hybridization and introgression. It is unclear how all this has occurred – but the results indicated high levels of gene flow. The relatively heavy corn pollen is unlikely to have reached Sierra Norte de Oaxaca on the wind, as the nearest known plantings of Bt corn are 100 km away. However, nobody has any idea how much transgenic seed may have been grown or transported illegally. Mexico banned the planting of transgenic-corn in 1998, but it is still imported from the USA. These data have seriously alarmed conservationists worldwide, who see their worst fears realized.

The valuable results of a multi-year, range-wide survey was obtained in the study of the potential for reproductive contact between cultivated and common sunflower (*Helianthus annuus*). The results indicated that the opportunity for crop-wild hybridization exists throughout the range of sunflower cultivation. Approximately two-thirds of all cultivated fields occurred in close proximity to, and flowered coincidentally with, common sunflower populations. In these populations, the phenological overlap was extensive, with 52–96% of all wilds flowering coincidentally with the adjacent cultivar field. Moreover, there was morphological evidence of hybridization in 10–33% of the populations surveyed within a given year. These findings indicated that crop-wild hybridization is likely across the range of sunflower cultivation in the USA (Burke *et al* 2002). The recent and detailed study documented gene flow on a landscape level from creeping bentgrass (*Agrostis stolonifera* L.), one of the first wind-pollinated, perennial, and highly outcrossing transgenic crops being developed for commercial use. Most of the gene flow occurred within 2 km in the direction of prevailing winds. The maximal gene flow distances observed were 21 km and 14 km in sentinel and resident plants, respectively, that were located in primarily non-agronomic habitats. The selectable marker used in these studies was the *CP4 EPSPS* gene derived from *Agrobacterium* spp. strain CP4 that encodes 5-enol-pyruvylshikimate-3-phosphate synthase and confers resistance to glyphosate herbicide. Evidence for gene flow to 75 of 138

sentinel plants of *A. stolonifera* and to 29 of 69 resident *Agrostis* plants was based on seedling progeny survival after spraying with glyphosate in greenhouse assays and positive TraitChek, PCR, and sequencing results (Watrud *et al* 2004).

Three field experiments were performed to quantify hybridization between multiple transformed oilseed rape lines and two wild relatives (*B. rapa* and *R. raphanistrum*) in multiple field conditions. The effect of crop frequency was determined under high and low crop to wild relative ratios. Hybridization rates within fields and at field margins were compared. Backcrossing rates of transgenic hybrids with the wild relative were quantified in two experimental locations in order to determine the incidence of introgression during subsequent generations. Together, these experiments are among the first to comprehensively characterize hybridization and backcrossing frequency among different transformation events, sites, and years. Gene flow of green fluorescent protein (GFP) and *Bt* transgenes was quantified in three field experiments using eleven independent transformed *Brassica napus* L. lines and the wild relatives, *B. rapa* and *R. raphanistrum*. Under a high crop to wild relative ratio (600 : 1), hybridization frequency with *B. rapa* differed among the individual transformed *B. napus* lines (ranging from ca. 4% to 22%), however, this difference could be caused by the insertion events or other factors, e.g. differences in the hybridization frequencies among the *B. rapa* plants. The average hybridization frequency over all transformed lines was close to 10%. No hybridization with *R. raphanistrum* was detected. Under a lower crop to wild relative ratio (180 : 1), hybridization frequency with *B. rapa* was consistent among the transformed *B. napus* lines at ca. 2%. Interspecific hybridization was higher when *B. rapa* occurred within the *B. napus* plot (ca. 37·2%) compared with plot margins (ca. 5·2%). No significant differences were detected among marginal plants grown at 1, 2, and 3 m from the field plot. Transgene backcrossing frequency between *B. rapa* and transgenic hybrids was determined in two field experiments in which the wild relative to transgenic hybrid ratio was 5–15 plants of *B. rapa* to 1 transgenic hybrid. As expected, ca. 50% of the seeds produced were transgenic backcrosses when the transgenic hybrid plants served as the maternal parent. When *B. rapa* plants served as the maternal parent, transgene backcrossing frequencies were 0·088% and 0·060%. Results demonstrated that transgene flow from many independent transformed lines of *B. napus* to *B. rapa* can occur under a range of field conditions, and that transgenic hybrids have a high potential to produce transgenic seeds in backcrosses (Halfhill *et al* 2004).

Also, it was found that pollen-mediated gene flow up to 31 m from Bt corn caused low to moderate Bt toxin levels in kernels of non-Bt corn plants. Immunoassays of non-Bt corn sampled from the field showed that the mean concentration of Bt toxin Cry1Ab in kernels and the percentage of kernels with Cry1Ab decreased with distance from Bt corn. The highest Bt toxin concentration in pooled kernels of non-Bt corn plants was 45% of the mean concentration in kernels from adjacent Bt maize plants. Authors suggested, that genes gene flow from transgenic crops has serious implications for pest resistance management. Variable Bt toxin production in seeds of refuge plants undermines the high-dose/refuge strategy and could accelerate pest resistance to Bt crops (Chilcutt and Tabashnik 2004).

Moreover, there is the recent report of the findings relevant to the evaluation of the risk of introgression of transgenic potato in its main centre of biodiversity, the central Andes. This is a centre of origin for the crop, with 130 wild potato species (*Solanum* L. section Petota Dumort) recognized in Peru and Bolivia. The transgenic nematode-resistant potato that express a cysteine proteinase inhibitor (cystatin) from rice seeds in roots controls potato cyst nematode (*Globodera* spp.) by impairing digestion of its dietary protein. For the establishing whether gene flow would occur from the transgenic potato field trials six wild species were selected on the basis of a preliminary survey of four agro-ecological zones in Peru to define examples of wild *Solanum* species that occur close to potato plots and five open pollination field trials were established in different agro-ecological zones at Puno, Junýn, Cajamarca and Cusco. Amplified fragment length polymorphism (AFLP) displayed that the progeny from each of three wild relatives with accessions in the trial included a few seedlings that were fathered by *S. tuberosum* cultivar group Andigenum. This confirmed that gene flow can occur from a cultivated potato to wild relatives in the field. Assuming that the nearest plant of the male parent identified by AFLP fingerprinting was the pollen donor, their mean distance was less than three plants from the pollen recipient (Celis *et al* 2004). It was consistent with early observations that 2% of seedlings were transgenic when the cross-pollination distance from non-transgenic females to transgenic pollen donors was 3 m and 24% were transgenic when the parents grew next to each other (McPartlan and Dale 1994).

(ii) *No*

Non-Bt corn was planted adjacent to Bt corn to determine the effects of Bt corn pollen falling on non-Bt plants for control of ECB larvae, *O. nubilalis* (Hubner). Field plots in Iowa and Kansas consisted of two center rows of Bt corn with eight rows of adjacent non-Bt corn on each side. In mid-September 1996 and 1997, ECB larvae and larval tunnels in the stalk and ear shank were counted. There were no significant differences in ECB numbers

across non-Bt rows and the slope of the regression line was not significantly different from zero. In a single plot in Iowa, however, fewer tunnels were observed in rows of corn that were closer to Bt corn. This site was isolated from natural infestations and probably does not reflected a typical field situation. The results suggested that Bt pollen has minimal or no control of ECB larvae in adjacent rows of non-Bt corn under natural conditions. Authors suggested, that "Bt pollen drifting onto adjacent non-Bt plants should not increase the risks related to resistance management" (Pilcher *et al* 2001).

4.5 *Will TPs be more competitive than corresponding non TPs*

According the worst case scenario the inevitable escape of transgenic pollen from cultivated fields will lead to the emergence of transgenic crop-wild plant hybrids in natural patches of wild plants. The fate of these hybrids and that of the transgene will depend on their ability to compete with their wild relatives. The possibility for increased fitness of transgenic wild relative × crop hybrid and backcross generations depends on the nature of the transgene. The serious concern is expressed also about the relative fitness of the HT plants compared to the susceptible when no herbicide is applied since this will largely determine the long-term fate of the HT seeds outside of the field (Purrington and Bergelson 1999). Thus, only highly advantageous transgenes will spread rapidly enough to have a substantial ecological impact. So, are there any facts, that TPs could more fittest than corresponding non TPs?

(i) *Yes*

Bt protected TPs: *Brassica napus* L. (oilseed rape) is of particular interest as a potential source for transgene escape. The crop is predominantly selfing with outcrossing averaging 30% (reviewed in Beckie *et al* 2003). The TP tested was Bt rapeseed *B. napus* insecticidal towards certain caterpillars including the diamondback moth *Plutella xylostella* L. and the corn earworm *Helicoverpa tea* Boddie. To simulate an escape of the transgenics from cultivation, a field experiment was performed in which Bt and non Bt rapeseed plants were planted in natural vegetation and cultivated plots and subjected to various selection pressures in the form of herbivory from insects. Only two Bt plants, survived the winter to reproduce in the natural-vegetation plots which were dominated by grasses such as crabgrass. However, in plots that were initially cultivated then allowed to naturalize, medium to high levels of defoliation decreased survivorship of non Bt plants relative to Bt plants and increased differential reproduction in favour of Bt plants. Thus, where suitable habitat is readily available, there is a likelihood of enhanced ecological risk associated with the release of certain transgene/crop combinations such as insecticidal rapeseed. The authors claimed, that "this is the first report of a field study demonstrating the effect of a fitness-increasing transgene in plants". (Stewart *et al* 1997). The level of transgene expression in crop × weed hybrids and the degree to which crop-specific genes are integrated into hybrid populations are important factors in assessing the potential ecological and agricultural risks of gene flow associated with genetic engineering. The average transgene zygosity and genetic structure of transgenic hybrid populations change with the progression of generations, and the GFP transgene is an ideal marker to quantify transgene expression in advancing populations. The homozygous T1 single-locus insert GFP/Bt transgenic canola (*Brassica napus*, cv Westar) with two copies of the transgene fluoresced twice as much as hemizygous individuals with only one copy of the transgene. These data indicated that the expression of the GFP gene was additive, and fluorescence could be used to determine zygosity status. Several hybrid generations (BC1F1, BC2F1) were produced by backcrossing various GFP/Bt transgenic canola (*B. napus*, cv Westar) and birdseed rape (*B. rapa*) hybrid generations onto *B. rapa*. Intercrossed generations (BC2F2 Bulk) were generated by crossing BC2F1 individuals in the presence of a pollinating insect (*Musca domestica* L.). The ploidy of plants in the BC2F2 Bulk hybrid generation was identical to the weedy parental species, *B. rapa*. AFLP analysis was used to quantify the degree of *B. napus* introgression into multiple backcross hybrid generations with *B. rapa*. The F1 hybrid generations contained 95–97% of the *B. napus*-specific AFLP markers, and each successive backcross generation demonstrated a reduction of markers resulting in the 15–29% presence in the BC2F2 bulk population. Average fluorescence of each successive hybrid generation was analysed, and homozygous canola lines and hybrid populations that contained individuals homozygous for GFP (BC2F2 bulk) demonstrated significantly higher fluorescence than hemizygous hybrid generations (F1, BC F1 and BC2F1). These data showed that the formation of homozygous individuals within hybrid populations increases the average level of transgene expression as generations progress. This phenomenon must be considered in the development of risk-management strategies.

The GFP gene demonstrated additive transgene expression in ten independent transformation events of canola. In all canola lines, homozygous individuals that contained two copies of the transgene locus fluoresced twice as much as hemizygous individuals above the background level of fluorescence (Halfhill *et al* 2003). Similarly, a *Bt* transgene that reduces insect herbivory in cultivated sunflowers (*Helianthus annuus* L.) also does so in wild sunflowers, leading to increased fecundity in one of the two sites studied (Snow *et al* 2003).

Later, the differential response of diamondback moth (*Plutella xylostella*) populations in eastern and western Canada to *Bt*-producing (GT) *B. napus* and the potential for enhanced fitness of GT *B. napus* and weedy GT *B. rapa* × *B. napus* hybrid populations (F1, BC1, BC2) were studied. Comparative bioassays using neonates and 4th instars showed that GT *B. napus* and GT *B. rapa* × *B. napus* hybrids are lethal to larvae from both populations. No measurable plant fitness advantage (reproductive dry weight) was observed for GT *B. napus* (crop) and GT *B. rapa* × *B. napus* hybrid populations at low insect pressure (1 larva per leaf). Although insect densities (> 10 larvae per leaf), vegetative plant weight was not significantly different for GT *B. napus* and non-GT *B. napus*, whereas reproductive plant weight and proportion of reproductive material were significantly higher in GT *B. napus*. Establishment of the *Bt* trait in wild *B. rapa* populations may also increase its competitive advantage under high insect pressure (Zhu *et al* 2004).

Little is known about the long-term persistence of transgenes from different transformation events. For example, transgenes that are located on the crop's C chromosomes may be lost during the process of introgression. The genetic behaviour of transgenes was investigated in backcross generations of wild *B. rapa* after nine GFP-Bt *B. napus* lines, named GT lines, were hybridized with three wild *B. rapa* accessions, respectively. Each backcross generation involved crosses between hemizygous GT plants and non-GT *B. rapa* pollen recipients. In some cases, sample sizes were too small to allow the detection of major deviations from Mendelian segregation ratios, but the segregation of GT: non-GT was consistent with an expected ratio of 1 : 1 in all crosses in the BC1 generation. Starting with the BC2 generation, significantly different genetic behaviour of the transgenes was observed among the nine GT *B. napus* lines. In some lines, the segregation of GT: non-GT showed a ratio of 1 : 1 in the BC2, BC3, and BC4 generations. However, in other GT *B. napus* lines the segregation ratio of GT: non-GT significantly deviated from 1 : 1 in the BC2 and BC3 generations, which had fewer transgenic progeny than expected, but not in the BC4 generation. Most importantly, in two GT *B. napus* lines the segregation of GT: non-GT did not fit into a ratio of 1 : 1 in the BC2, BC3 or BC4 generations due to a deficiency of transgenic progeny. For these lines, a strong reduction of transgene introgression was observed in all three *B. rapa* accessions. These findings imply that the genomic location of transgenes in *B. napus* may affect the long-term persistence of transgenes in *B. rapa* after hybridization has occurred (Zhu *et al* 2004).

Mixed stands of wild plants and first-generation hybrids were grown under different conditions of herbivore pressure and density, with Bt oilseed rape (*B. napus*) as the crop and *B. rapa* as the wild recipient. Biomass and fitness components were measured from plant germination to the germination of their offspring. The frequency of transgenic seedlings in the offspring generation was estimated using the green fluorescent protein marker. The biomass of F(1) Bt-transgenic hybrids relative to that of wild-type plants was found to be sensitive to both plant density and herbivore pressure, but herbivore pressure appeared as the major factor enhancing their relative fitnesses. In the absence of herbivore pressure, Bt hybrids produced 6·2-fold fewer seeds than their wild neighbours, and Bt plant frequency fell from 50% to 16% within a single generation. Under high herbivore pressure, Bt hybrids produced 1·4-fold more seeds, and Bt plant frequency was 42% in the offspring generation. It was concluded that high-density patches of highly damaged wild plants are the most vulnerable to Bt-transgene invasion. They should be monitored early to detect potential transgene spread (Vacher *et al* 2004).

PI protected potato: Introgression of a transgene from transgenic potato that express a cysteine proteinase inhibitor (cystatin) might confer resistance to nematodes on wild relatives that are currently susceptible to *Globodera* spp. It was found that this nematode did reproduce on five of six wild *Solanum* species studied with an increase in number ranging from approx. 4·38-fold for *S. acaule* to approx. 2·87 for *Solanum raphanifolium*. The exception was *S. sparsipilum* (approx. 0·89-fold). Susceptible native and improved cultivars were larger with more substantial root systems than their wild relatives, and they supported higher multiplication of *Globodera* (approx. 19·8-fold). The possibility that a stably introgressed transgene for pest resistance might benefit a wild relative growing near a crop species requires examination on a case-by-case basis (Celis *et al* 2004).

(ii) *No*

HT transgenic plants: A comparison of fitness of the sulfonylurea-resistant line of white-chicory regenerated with that of the supposedly isogenic susceptible biotype was made. The plants were grown in experimental plots at a range of densities in a replacement series. The reproductive output of the plants decreased with increasing density but no significant difference was found between the two lines for any vegetative or reproductive trait at any density. This suggested that no cost is associated with the mutation causing the resistance and that the resistance gene would not be selected against if it escaped to populations of wild chicories (Lavigne *et al* 1995).

The transgenic plant was *Arabidopsis thaliana* expressing a mutant acetolactate synthase gene that confers resistance to the herbicide, chlorsulfuron. It was revealed

34% reduction in the lifetime seed production of transgenic, herbicide resistant *A. thaliana* relative to their susceptible null-segregants. The experimental design allowed to conclude "that this fitness cost of resistance is caused by the pleiotropic effect of the cloned acetolactate synthase gene rather than other potential costs associated with the plasmid or mutational changes induced by plant transformation". In general, the cost of resistance was attributed to the presence of the resistance gene rather than an increase in gene dosage (Bergelson *et al* 1996).

In general, glyphosate, the active ingredient in the herbicide roundup, has increased dramatically in use over the past decade and constitutes a potent anthropogenic source of selection. In the southeastern United States, weedy morning glories have begun to develop tolerance to glyphosate, representing a unique opportunity to examine the evolutionary genetics of a novel trait. Genetic variation for tolerance, indicating the potential for the population to respond to selection by glyphosate was found recently. However, the following significant evolutionary constraint exists: in the absence of glyphosate, tolerant genotypes produced fewer seeds than susceptible genotypes. The combination of strong positive directional selection in the presence of glyphosate and strong negative directional selection in its absence may indicate that the selective landscape of land use could drive the evolutionary trajectory of glyphosate tolerance (Baucom and Mauricio 2004).

Other TPs: The fitness effects of a transgene conferring resistance to white mold (*Sclerotinia* sclerotiorum) in sunflower (*H. annuus*) was examined. An oxalate oxidase (OxOx) gene was used to enhance white mold resistance in cultivated sunflower presumably by degrading oxalic acid, which contributes to white mold pathogenicity. Presence or absence of the OxOx transgene had no effect on seed output, indicating that there was no cost of resistance in the absence of a pathogen challenge. In terms of infection rates, the OxOx gene did provide protection against white mold. The transgene did not, however, have any effect on seed output after inoculation. Though the transgene provided protection against white mold infection, it had no effect on reproductive output. The results suggest that the OxOx transgene will do little more than diffuse neutrally after its escape. This is especially true because the experiment simulated the worst case scenario, in which early generation hybrids faced a severe pathogen challenge. The auhors noted, that this work was performed within a single season and on a single genetic background and the results may not be generalizable (Burke and Rieseberg 2003).

The growth, yield, population dynamics and competitive ability of transgenic *Trifolium subterraneum* sub sp. *subterraneum* cv. *Leura* (subclover) expressing a nutritive sunflower seed albumin (*ssa*) gene was compared with the equivalent non-transgenic commercial line in a glasshouse competition trial. Plants were grown in low-fertility soil typical of unimproved native southeastern Australian grasslands. The survivorship, seed production rate, seed germination rate, seed weight, dry weight yield and the intrinsic rate of population increase of plants grown in mixtures and monocultures over a range of densities (250 to 2000 plants m^2), were measured and intragenotypic and intergenotypic competition coefficients for each line were also determined. There were no significant differences between transgenic and non-transgenic plants in any of the measured variables except survivorship; transgenic plants had a significantly lower survival rate than non-transgenic plants when grown at high densities. However, density-dependent effects were observed for all measured variables, and in all models plant density affected the response variables more than the presence of the transgene. It was concluded that the *ssa* gene construct appears to confer no advantage to transgenic *T. subterraneum* cv. Leura growing in mixed or pure swards under the fertility and density regimes examined in the trial. The data also suggested that transgenic subterranean clover expressing the *ssa* gene is unlikely to exhibit a competitive advantage over associated non-transgenic commercial cultivars when grown in dense swards in low-fertility pastures (Godfree *et al* 2004).

Transgenic pollen with a visible marker gene could be useful to monitor the movement of transgenic pollen provided there are no negative physiological or fitness effects of expressing such a gene. The fitness of *Nicotiana tabacum* cv. Xanthi pollen expressing the marker gene GFP was measured. Average pollen tube germination frequencies and pollen tube growth rates *in vitro* were measured in three different types of plants: (i) plants producing GFP in pollen cells only (LAT59-GFP); (ii) plants synthesizing GFP under the control of a constitutive promoter (CaMV 35S) in which no GFP was produced in pollen; and (iii) non-transgenic plants. Pollen synthesizing the GFP protein did not differ significantly in average pollen germination frequencies from pollen without GFP. Average pollen tube growth rates over a 5 h period did not differ significantly between transgenic and non-transgenic types. Overall, GFP expression in pollen grains of tobacco was not found to have an effect on pollen fitness under the controlled experimental conditions of this study (Hudson and Stewart 2004).

4.6 *Will cultivation of the HT transgenic plants lead to formation of the herbicide tolerant superweed?*

Varieties with HT traits account for the majority of transgenic crops and have shown the most rapid adoption by North American producers, followed by insect-resistant varieties. The rapid adoption of herbicide-tolerant crops

is mainly due to the introduction of roundup ready crops in 1996 which allowed the use of glyphosate (roundup) as a postemergence herbicide at any stage of growth (Carpenter and Gianessi 2000). One element of the current public debate about HT plants is that gene flow from transgenic cultivars into surrounding weed populations will lead to more problematic weeds, particularly for traits such as herbicide resistance. Is this risk considerable?

(i) *Yes*

Recent experiments with HT canola (*B. napus*) repeatedly confirm that genes and transgenes will flow and hybrids will form if certain conditions are met. These include sympatry with a compatible relative (weedy, wild or crop), synchrony of flowering, successful fertilization and viable offspring. The chance of these events occurring is real; however, it is generally low and varies with species and circumstances. Plants of the same species (non-transgenic or with a different HT transgene) in neighbouring fields may inherit the new HT gene, potentially generating plants with single and multiple HT. For canola, seed losses at harvest and secondary dormancy ensures the persistence over time of the HT trait(s) in the seed bank, and the potential presence of crop volunteers in subsequent crops. Although canola has many wild/weedy relatives, the risk of gene flow is quite low for most of these species, except with *B. rapa*. Introgression of genes and transgenes in *B. rapa* populations occurs with apparently little or no fitness costs. Consequences of HT canola gene flow for the agro-ecosystem include contamination of seed lots, potentially more complex and costly control strategy, and limitations in cropping system design. Consequences for non-agricultural habitats may be minor but appear largely undocumented (Legere 2005).

The aim of model GENESYS was to rank cropping systems according to their risk of gene escape from transgenic HT winter oilseed rape cultivars to rapeseed volunteers. The model integrated the effects of crop succession and crop management at the level of a region. The first part of the model described the temporal evolution of rape seed volunteers in a field, using an annual life-cycle comprising stages such as seed bank, seedlings, adult plants, rowers or freshly produced seeds. The relationships between the various stages depend on the crops grown each year and the cultivation techniques (stubble breaking, soil tillage, sowing date and density, herbicides, cutting and harvesting). Parameter values were either deduced from existing models and literature, or estimated from experimental studies and field surveys (Colbach *et al* 2001). The next was a model for gene flow incorporating exponential distance and directional effects to be applied to wind pollinated species. This model is applied to data on gene flow in experimental plots of *Agrostis stolonifera* L. (creeping bentgrass), which assessed gene flow from transgenic plants resistant to the herbicide glufosinate to surrounding non-transgenic plants. The results shown that although pollen dispersal can be limited in some sites, it may be extensive in others, depending on local conditions such as exposure to wind. Thus, hybridization under field conditions is likely to occur. Given the nature of the herbicide resistance trait it was regarded that this trait as unlikely to persist in the absence of herbicide, and suggested that the ecological consequences of such gene flow are likely to be minimal (Meagher *et al* 2003).

4.7 *How to reduce a risk of pollen mediated introgression?*

Several strategies have been proposed for creating transgenic cultivars from which transgene escape to wild relatives would seem unlikely; for example, to impede escape through pollen, a transgene could be inserted into chloroplast DNA (cpDNA), which in many crops is rarely transmitted through pollen. None of these strategies would be failsafe; for example, the rate of cpDNA transmission through pollen may be low but non-zero in many crops. The main questions are: how the probability distribution of escape time depends on the rates of pollen and seed flow from the crop to wild populations, the number and sizes of the wild populations, the selection coefficient for the transgene, and a leakage parameter characteristic of the strategy, for example, the rate of cpDNA transmission through pollen?. It was estimated that even with a leakage parameter as small as 10^{-3}, the probability of escape within as few as 10 generations could be appreciable (Haygood *et al* 2004).

As it was noted above, cultivation of transgenic potato that provide resistance to nematodes in its main centre of biodiversity, resulted transgenes gene flow to wild relatives growing near potato crops (Celis *et al* 2004). If stable introgression were to result, the fitness of these wild species could be altered. Therefore the male sterile cultivar Revolucion was transformed to provide a transsgenic nematode-resistant potato to evaluate the benefits that this provides until the possibility of stable introgression to wild relatives is determined. The authors suggested, that "scientific progress is possible without compromise to the precautionary principle" (Celis *et al* 2004).

Biological confinement strategies (e.g. male sterility, gene insertion into targeted chromosomes or chromosome sites or into organelles) are of interest to try to restrict gene flow. Transgenic HT *B. napus* can be easily crossed with wild *B. rapa*. The special study was designated, by means of population genetics, to study the fate of a transgene escape from *B. napus* to *B. rapa*. Three

models were proposed to survey the change in gene frequency during successive back cross processes by considering selection pressures against aneuploids, against herbicide-susceptible individuals, and by considering A-C intergenomic recombination and the effect of genetic drift. The transmission rate of an A-chromosome gene through an individual to the next generation was 50%, irrespective of the chromosome number; while that of a C-chromosome transgene varied from 8·7% to 39·9%, depending on the chromosome number of the individual used in the backcross. Without spraying herbicide, the frequency of an A-chromosome gene was 50% in the BC, generation, and decreased by 50% with the advance of each backcross generation; that of a C-chromosome gene was around 39·9% in BC1, 7·7% in BC2, 1·2% in BC3 and 0·1% in the BC4 generation. Under the selection pressure against herbicide-susceptible individuals, the frequency of a transgene reached a stable value of about 5·5% within six generations of successive backcrossings. It was suggested that the transgene integrated on a C-chromosome (or better on a cytoplasm genome) is safer than that on an A-chromosome. It was proposed, that transgenic cultivars should be cultivated rotationally by year(s) with other non-transgenic varieties in order to reduce the transfer of the transgene to wild *B. rapa* species (Lu *et al* 2002). Since the plastid transgenes are not transmitted by pollen cloning transgenes in an organelles genomes could be used for containment of transgenes in transgenic crops plants. The amplification of crylA(c) toxin coding sequence in chloroplasts of tobacco up to similar to 10,000 copies per cell resulted in the accumulation of an unprecedented 3–5% of the soluble protein in tobacco leaves as protoxin. The plants were extremely toxic to larvae of *Heliothis virescens*, *Helicoverpa zea*, and *Spodoptera exigua* (McBride *et al* 1995). When transgenic tobacco leaves expressing Cry2Aa2 protoxin in chloroplasts were fed to susceptible, CrylA-resistant (20,000 to 40,000-fold) and Cry2Aa2-resistant (330- to 393-fold) tobacco budworm *H. virescens*, cotton bollworm *H. tea*, and the beet armyworm *S. exigua*, 100% mortality was observed against all insect species and strains (Kota *et al* 1999). Allso, there is the possibility to clone transgens in the plant chloroplast genome without the use of antibiotic markers (Daniell *et al* 2001).

5. Can TPs increase a biodiversity?

The development of cotton pest management practices in China has followed a pattern seen for many crops that rely heavily on insecticides. *H. armigera* resistance to chemical pesticides resulted in unprecedented pest densities of the early 1990s. Field trials of *H. armigera* resistant transgenic cotton were conducted in the early 1990s in China. *Cry1A* cotton and *Cry1A + CpTI* (cowpea trypsin inhibitor) cotton were approved for planting by the Chinese government in 1997 and 1999, respectively. By 2003, five *Cry1A* cotton varieties and four *Cry1A+CpTI* cotton varieties from the Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, and four *Cry1Ac* cotton varieties from the Monsanto Company (St. Louis, Missouri, USA) were planted commercially. Plantings of Bt cotton totaled less than 0·1 million ha in 1997, but expanded rapidly to 1·1 million ha in 2000 and 2·8 million ha in 2003. This represented 58% of the total cotton acreage of 4·8 million ha in 2003. Indirect effects of TPs on nontarget pest species may be positive owing to the removal of disruptive pesticides or negative owing to the increase in density of natural enemies resulting from a decrease in chemical use.

Field experiments on population dynamics of cotton aphid in Bt cotton fields indicated that planting Bt cotton efficiently prevents a resurgence of cotton aphids caused by insecticide use for control of cotton bollworm. On the other hand, an investigation of the seasonal dynamics of mixed populations of mirids in different cotton fields demostrated that mirid density was significantly higher on nonsprayed Bt cotton than on sprayed non-Bt cotton owing to a reduction in the number of insecticide applications against *H. armigera*. This suggesed that the mirids have become key insect pests in Bt cotton fields, and their damage to cotton could increase further with the expansion of Bt cotton-growing areas if no additional control measures are adopted. In general, the pest management tactics associated with Bt cotton have resulted in a drastic reduction in insecticide use, which usually results in a significant increase in populations of beneficial insects and thus contributes to the improvement of the natural control of some pests. Results obtained in recent years in China indicated that the predator levels in *Bt* cotton fields are significantly higher than those in conventional cotton fields where insecticide has been used for control of the cotton bollworm, *H. armigera* (Wu and Guo 2004).

5.1 *Can cultivation of Bt cotton increase populations of natural enemies?*

Transgenic Bt cotton may affect natural enemies indirectly through the removal of eggs, larvae, and pupae of lepidopterans that serve as food sources for parasitic and predatory arthropods.Considerable reduction in the number of insecticide applications is another important factor that regulates population dynamics of natural enemies. Field trials demonstrated that by midseason the population density of predators, such as lady beetles (*Coccinella septempunctata*, *Leis axyridis*, and *Propylaea japonica*), lacewings (*Chrysopa sinica*, *C. septempunctata*, *C. shansiensis*, and *C. formosa*), spiders (*Erigonidium graminicolum* and *Misumenops tricuspidata*), and *Orius similis*,

in Bt cotton are significantly higher than those in conventional cotton fields treated with insecticides for control of *H. armigera*. However, the population densities of parasitic wasps (*Singa hamata, Trichogramma confusum, Microplitis mediator, Campoletis chlorideae,* and *Litomastix* sp.) decreased significantly owing to poor quality and lower density of *H. armigera* in Bt cotton fields (Wu and Guo 2004).

5.2 *Can cultivation of Bt cotton increase diversity of Arthropods?*

The population density of parasitic wasps which parasitise on *H. armigera* larvae decreased considerably due to the lower density and poor quality of *H. armigera* larvae in *Bt* cotton fields. As the predator population increases, the outbreak of cotton aphid in mid-season is effectively controlled, while the mirids become key insect pests in *Bt* cotton fields because of a reduced number of insecticides used against *H. armigera*. The diversity of the arthropod community in *Bt* cotton fields was higher than that in conventional cotton, suggesting that *Bt* cotton is highly favourable for integrated management of cotton pests. An assessment on the impact of *Bt* cotton pollen on two important economic insects, the Chinese tussah silkworm, *Antnaea perngicuerin* (*Antheraea pernyi*) and the silkworm, *Bombyx mori*, was conducted, from which it was concluded that the adverse effect is negligible (Wu *et al* 2003).

6. Can cultivation of Bt crops improve human health?

One on the most serious negative effects of the large scale usage of chemical herbicides is their toxicity to mammals. There is proposed an environmental indicator based on a standardized, well-known acute mammalian toxicity measure, the LD_{50} dose for rats. This indicator was used to compare an environmental effect of the cultivation of HT soybeans to the cultivation of non HT soybeans for over 1400 US Midwest farms. The results suggested that HT soybean seed technology is more environmental friendly than non-HT technology for all farms in the dimension of acute mammalian toxicity. The effect is generally more pronounced in the south where a longer growing season makes overall weed pressure more serious and presents soybean growers with a greater variety of weed species (Nelson and Bullock 2003).

Moreoevr, there is the first evidence of a direct link between the adoption of a TPs and improvements in human health: the estimation of the impact of Bt cotton adoption on pesticide use from data from a survey of cotton farmers in northern China, 1999–2001, showed that Bt cotton adoption reduced pesticide use. Assessment of a health-production function showed that predicted pesticide use had a positive impact on poisoning incidence. Taken together, these results indicated that the adoption of Bt cotton an substantially reduce the risk and the incidence of poisonings (Hossain *et al* 2004).

7. Summary

The review of the available experimental data concerning direct environmental risks possessing by TPs display that results of laboratory, greenhouse and filed testing of different TPs cultivated in different conditions are difficult to compare, risky to extrapolate but easy to over generalize. Overall, it seems, that studies on nontarget TPs feeding insects and beneficial species that has accompanied the long-term and wide-scale use of TPs not detected serious negative ecological consequences. It is believed, that TPs have little impact on soil biota such as earthworms, collembolans, and general soil microflora. In fact it is logical given the lability of transgenic proteins in biological systems relative to chemical pesticides and herbicides and suggests that the degree of exposure of natural enemy species to insecticidal proteins will not be considerable.

The main concerns dealing with the negative ecological impacts of TPs could be associated with (i) non target effects of insecticidal TPs possessing a broad spectrum of toxicity, with (ii) risk of introgression, followed by (iii) increased fitness of formed hybrids and their invasiveness.

The serious problems of emergence and spread of target and non target organisms, resistant to insecticidal TPs was not the scope of this work.

The conclusion which could made on the basis of available data dealing with indirect ecological consequences of large scale implementation of inseciticidal and HT. TPs is that they improve the corresponding environments because they considerably reduce the load of chemical insecticides and herbicides.

In general, risk assessments and potential adverse environmental impacts of TPs should be evaluated and interpreted in comparison with that of corresponding non TPs cultivated under the treatment of corresponding chemical insecticides and herbicides.

Acknowlegements

We apologize to many authors for not citing them owing to limitation of space. This work was supported by grant ISTC N 2877P.

Refereneces

Al-Deeb M A, Wilde G E, Blair J M and Todd T C 2003 Effect of Bt corn for corn rootworm control on nontarget soil microarthropods and nematodes; *Environ. Entomol*. **32** 859–865

Asao H, Arai S and Nishizawa Y 2003 Environmental risk evaluation of transgenic strawberry expressing a rice chitinase gene; *Seibutsu Kogaku Kaishi* **81** 57–63

Astrid T, Groot A T and Dicke M 2002 Insect-resistant transgenic plants in a multi-trophic context; *Plant J.* **31** 387– 406

Baucom R S and Mauricio R 2004 Fitness costs and benefits of novel herbicide tolerance in a noxious weed; *Proc. Natl. Acad. Sci. USA* **101** 13386–13390

Baur M E and Boethel D J 2003 Effect of Bt cotton expressing Cry1A(c) on the survival and fecundity of two hymenopteran parasitoids (Braconidae, Encyrtidae) in the laboratory; *Biol. Control* **26** 325–332

Beckie H J Warwick S I Nair H and Sequin-Swartz G 2003 Gene flow in commercial fields of herbicide-resistant oilseed rape (*Brassica napus*); *Ecol. Appl.* **13** 1276–1294

Bell H A, Down R E, Fitches E C, Edwards J P and Gatehouse A M R 2003 Impact of genetically modified potato expressing plant-derived insect resistance genes on the predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae); *Biocontrol Sci. Technol.* **13** 729–741

Bell H A, Fitches E C, Marris G C, Bell J, Edwards J P, Gatehouse J A and Gatehouse A M R 2001 Transgenic GNA expressing potato plants augment the beneficial biocontrol of *Lacanobia oleracea* (Lepidoptera; Noctuidae) by the parasitoid *Eulophus pennicornis* (Hymenoptera; Eulophidae); *Transgenic Res.* **10** 35–42

Bergelson J, Purrington C B, Palm C J and LopezGutierrez J C 1996 Costs of resistance: A test using transgenic *Arabidopsis thaliana*; *Proc. R. Soc. London Ser. B.* **263** 1659–1663

Bernal C C, Aguda R M and Cohen M B 2002 Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis; Entomol. Exp. Appl.* **102** 21–28

Bernal J S, Griset J G and Gillogly P O 2002 Impacts of developing on Bt maize-intoxicated hosts on fitness parameters of a stem borer parasitoid; *J. Entomol. Sci.* **37** 27–40

Birch A N E, Geoghegan I E, Majerus M E N, McNicol J W, Hackett C A, Gatehouse A M R and Gatehouse J A 1999 Tritrophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance; *Mol. Breed* **5** 75–83

Bitzer R J, Buckelew L D and Pedigo L P 2002 Effects of transgenic herbicide-resistant soybean varieties and systems on surface-active springtails (*Entognatha*: *collembola*); *Environ. Entomol.* **31** 449–461

Borisjuk N V, Borisjuk L G, Logendra S, Petersen F, Gleba Y and Raskin I 1999 Production of recombinant proteins in plant root exudates; *Nat. Biotechnol.* **17** 466–469

Bouchard E, Cloutier C and Michaud D 2003a Oryzacystatin I expressed in transgenic potato induces digestive compensation in an insect natural predator via its herbivorous prey feeding on the plant; *Mol. Ecol.* **12** 2439–2446

Bouchard E, Michaud D and Cloutier C 2003b Molecular interactions between an insect predator and its herbivore prey on transgenic potato expressing a cysteineproteinase inhibitor from rice; *Mol. Ecol.* **12** 2429–2437

Brodsgaard H F, Brodsgaard C J, Hansen H and Lovei G L 2003 Environmental risk assessment of transgene products using honey bee (*Apis mellifera*) larvae; *Apidologie* **34** 139–145

Bruinsma M, Kowalchuk G A and van Veen J A 2003 Effects of genetically modified plants on microbial communities and processes in soil; *Biol. Fertil. Soils* **37** 329–337

Brusetti L, Fracia P, Bertolini C, Pagliuca A S B *et al* 2004 Bacterial communities associated with the rhizosphere of transgenic Bt 176 maize (*Zea mays*) and its non-transgenic counterpart; *Plant Soil* **218** 137–144

Buckelew L D, Pedigo L P, Mero H M, Owen M D K and Tylka G L 2000 Effects of weed management systems on canopy insects in herbicide-resistant soybeans; *J. Econ. Entomol.* **93** 1437–1443

Burgess E P J, Lovei G L, Malone L A, Nielsen I W, Gatehouse H S and Christeller J T 2002 Prey-mediated effects of the protease inhibitor aprotinin on the predatory carabid beetle *Nebria brevicollis*; *J. Insect Physiol.* **48** 1093–1101

Burgess E P J, Malone L A and Christeller J T 1996 Effects of two proteinase inhibitors on the digestive enzymes and survival of honey bees (*Apis mellifera*); *J. Insect Physiol.* **42** 823–828

Burgess E P J, Malone L A, Christeller J T, Lester M T, Murray C *et al* 2002 Avidin expressed in transgenic tobacco leaves confers resistance to two noctuid pests, *Helicoverpa armigera* and *Spodoptera litura; Transgenic Res.* **11** 185–198

Burke J M, Gardner K A and Reisenberg L H 2002 The potential for gene flow between cultivated and wild sunflower (*Helianthus annuus*) in the United Sates American; *J. Bot.* **89** 1550–1552

Burke J M and Rieseberg L H 2003 Fitness Effects of Transgenic Disease Resistance in Sunflowers; *Science* **300** 1250–1253

Cao J, Ibrahim H, Garcia J J, Mason H, Granados R R and Earle E D 2002 Transgenic tobacco plants carrying a baculovirus enhancing gene slow the development and increase the mortality of *Trichoplusia ni* larvae; *Plant Cell Reprod.* **21** 244–250

Carpenter J and Gianessi L 2000 Herbicide use on Roundup Ready crops; *Science* **287** 803–804

Celis C, Scurrah M, Cowgill S, Chumbiauca S, Green J, Franco J, Main G, Kiezebrink D, Visser R G and Atkinson H J 2004 Environmental biosafety and transgenic potato in a centre of diversity for this crop; *Nature* (*London*) **432** 222–225

Chilcutt C F and Tabashnik B E 2004 Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize; *Proc. Natl. Acad. Sci. USA* **101** 7526–7529

Colbach N, Clermont-Dauphin C and Meynard J M 2001 GENESYS: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers-I. Temporal evolution of a population of rapeseed volunteers in a field; *Agric. Ecosyst. Environ.* **83** 235–253

Conko G 2003 Safety, risk and the precautionary principle: rethinking precautionary approaches to the regulation of transgenic *plants; Transgenic Res.* **12** 639–647

Couty A, de la Vina G, Clark S J, Kaiser L, Pham-Delegue M H and Poppy G M 2001a Direct and indirect sublethal effects of *Galanthus nivalis* agglutinin (GNA) on the development of a potato-aphid parasitoid, *Aphelinus abdominalis* (Hymenoptera: Aphelinidae); *J. Insect Physiol.* **47** 553–561

Couty A Down R E Gatehouse A M R Kaiser L, Pham-Del'egue M H and Poppy G M 2001b Effects of artificial diet containing GNA and GNA-expressing potatoes on the development of the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae); *J. Insect Physiol.* **47** 1357–1366

Cowgill S E and Atkinson H J 2003 A sequential approach to risk assessment of transgenic plants expressing protease inhibitors: effects on non target herbivorous insects; *Transgenic Res.* **12** 439–449

Cowgill S E, Bardgett R D, Kiezebrink D T and Atkinson H J 2002a The effect of transgenic nematode resistance on non-target organisms in the potato rhizosphere; *J. Appl. Ecol.* **39** 915–923

Cowgill S E, Wright C and Atkinson H J 2002b Transgenic potatoes with enhanced levels of nematode resistance do not have altered susceptibility to nontarget aphids; *Mol. Ecol.* **11** 821–827

Crecchio C and Stotzky G 1998 Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. kurstaki bound to humic acids from soil; *Soil Biol. Biochem.* **30** 463– 470

Crecchio C and Stotzky G 2001 Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. kurstaki bound on complexes of montmorillonite-humic acids-Al hydroxypolymers; *Soil Biol. Biochem.* **33** 573–581

Daniell H, Muthukumar B and Lee S B 2001 Marker tree transgenic plants: engineering the chloroplast genome without the use of antibiotic selection; *Curr. Genet.* **39** 109–116

de Vries J, Heine M, Harms, K and Wackernagel W 2003 Spread of Recombinant DNA by Roots and Pollen of Transgenic Potato Plants, Identified by Highly Specific Biomonitoring Using Natural Transformation of an *Acinetobacter* sp.; *Appl. Environ. Microbiol*. **69** 4455–4462

Dinel H, Schnitzer M, Saharinen M, Meloche F, Par'e T *et al* 2003 Extractable soil lipids and microbial activity as affected by Bt and non-Bt maize grown on a silty clay loam soil; *J. Environ. Sci. Health* **B38** 211–219

Donegan K K, Palm C J, Fieland V J, Porteous L A, Ganio L M *et al* 1995 Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin; *Appl. Soil Ecol*. **2** 111–124

Donegan K K, Schaller D L, Stone J K, Ganio L M, Reed G *et al* 1996 Microbial populations, fungal species diversity and plant pathogen levels in field plots of potatoes expressing the *Bacillus thuringiensis* var. *tenebrionis* endotoxin; *Transgenic Res*. **5** 25–35

Donegan K K, Seidler R J, Fieland V J, Schaller D L, Palm C J *et al* 1997 Decomposition of genetically engineered tobacco under field conditions: persistence of the proteinase inhibitor I product and effects on soil microbial respiration and protozoa, nematode and arthropod populations; *J. Appl. Ecol*. **4** 767–777

Donegan K K, Seidler R J, Doyle J D, Porteous L A, Digiovanni G, Widmer F and Watrud L S 1999 A field study with genetically engineered alfalfa inoculated with recombinant *Sinorhizobium meliloti*: effects on the soil ecosystem; *J. Appl. Ecol.* **36** 920–936

Down R E, Ford L, Woodhouse S D, Davison G M, Majerus M E, Gatehouse J A and Gatehouse A M 2003 Tritrophic interactions between transgenic potato expressing snowdrop lectin (GNA), an aphid pest (peach-potato aphid; *Myzus persicae* (Sulz.) and a beneficial predator (2-spot ladybird; *Adalia bipunctata* L.); *Transgenic Res*. **12** 229–241

Duan J J, Head G, McKee M J, Nickson T E, Martin J W and Sayegh F S 2002 Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata; Entomol. Exp. Appl.* **104** 271–280

Dunfield K E and Germida J J 2003 Seasonal Changes in the Rhizosphere Microbial Communities Associated with Field-Grown Genetically Modified Canola (*Brassica napus*); *Appl. Environ. Microbiol.* **69** 7310–7318

Dunfield K E and Germida J J 2004 Impact of genetically modified crops on soil- and plant-associated microbial communities; *J. Environ. Qual*. **33** 806–815

Dutton A, Klein H, Romeis J and Bigler F 2002 Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea; Ecol. Entomol.* **27** 441–447

Dutton A, Klein H, Romeis J and Bigler F 2003a Prey-mediated effects of *Bacillusthuringiensis* spray on the predator *Chrysoperla carnea* in maize; *Biol. Control* **26** 209–215

Dutton A, Romeis J and Bigler F 2003b Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study; *BioControl* **48** 611–636

Escher N, Kach B and Nentwig W 2000 Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda); *Basic Appl. Ecol*. **1** 161–169

Ferry N, Jouanin L, Ceci L R, Mulligan E A, Emami K, Gatehouse J A and Gatehouse A M 2005 Impact of oilseed rape expressing the insecticidal serine protease inhibitor, mustard trypsin inhibitor-2 on the beneficial predator *Pterostichus madidus*; *Mol. Ecol*. **14** 337–349

Giovannetti M 2003 The ecological risks of transgenic plants; *Riv. Biol*. **96** 207–223

Glandorf D C M, Bakker P and VanLoon L C 1997 Influence of the production of antibacterial and antifungal proteins by transgenic plants on the saprophytic soil microflora; *Acta Bot. Neerl.* **46** 85–104

Godfree R C, Woods A G, Burdon J J and Higgins T J V 2004 Growth, fecundity and competitive ability of transgenic *Trifolium subterraneum* subsp. subterraneum cv. Leura expressing a sunflower seed albumin gene; *Hereditas* **141** 1–16

Graham J, Gordon S C, Smith K, McNicol R J and McNicol J W 2002 The effect of the Cowpea trypspin inhibitor in strawberry on damage by vine weevil under field conditions; *J. Hortic. Sci. Biotechnol.* **77** 33–40

Griffiths B S, Geoghegan I E and Robertson W M 2000 Testing genetically engineered potato, producing the lectins GNA and Con A, on non-target soil organisms and processes; *J. Appl. Ecol*. **37** 159–170

Halfhill M D, Millwood R J, Weissinger A K, Warwick S I and Stewart C N 2003 Additive transgene expression and genetic introgression in multiple green-fluorescent protein transgenic crop x weed hybrid generations; *Theor. Appl. Genet*. **107** 1533–1540

Halfhill M D, Zhu B, Warwick S I, Raymers P L, Millwood R J, Weissinger A K and Stewart C N Jr 2004 Hybridization and backcrossing between transgenic oilseed rape and two related weed species under field conditions; *Environ. Biosafety Res*. **3** 73–81

Haygood R, Ives A R and Andow D A 2004 Transgene containment, transgene escape, transgenic crops, wild relatives; *Ecol. Lett.* **7** 213–220

Head G, Surber J B, Watson J A, Martin J W and Duan J J 2002 No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use; *Environ. Entomol.* **31** 30–36

Hilbeck A 2001 Implications of transgenic, insecticidal plants for insect and plant biodiversity; *Perspect. Plant. Ecol. Evol. Syst.* **4** 43–61

Hilbeck A, Baumgartner M, Fried P M and Bigler F 1998 Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae); *Environ. Entomol.* **27** 480–487

Hokanson S C, Grumet R and Hancock J F 1997 Effect of border rows and trap/donor ratios on pollen-mediated gene movement; *Ecol. Appl.* **7** 1075–1081

Hopkins D W and Gregorich E G 2003 Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize; *Eur. J. Soil Sci.* **54** 793–800

Hossain F, Pray C E, Lu Y, Huang J, Fan C and Hu R 2004 Genetically modified cotton and farmers' health in China; *Int. J. Occup. Environ. Health* **10** 296–303

Hudson L C and Stewart C N Jr 2004 Effects of pollen-synthesized green fluorescent protein on pollen grain fitness; *Sex Plant Reprod.* **17** 49–53

Jaffe G 2004 Regulating transgenic crops: a comparative analysis of different regulatory processes; *Transgenic Res.* **13** 5–19

Jasinski J R, Eisley J B, Young C E, Kovach J and Willson H 2003 Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio; *Environ. Entomol.* **32** 407–413

Jesse L C H and Obrycki J J 2000 Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly; *Oecologia* **125** 241–248

Jesse L C H and Obrycki J J 2002 Assessment of the non-target effects of transgenic Bt corn pollen and anthers on the milkweed tiger moth; *Euchatias egle* Drury (Lepidoptera: Arctiidae); *J. Kans. Entomol. Soc.* **75** 55–58

Kaplinsky N, Braun D, Lisch D, Hay A, Hake S and Freeling M 2002 Biodiversity (Communications arising): maize transgene results in Mexico are artefacts; *Nature* (*London*) **416** 601–602

Kota M, Daniell H, Varma S, Garczynski S F, Gould F and Moar W J 1999 Overexpression of the *Bacillus thuringiensis* (Bt) Cry2Aa2 protein in chloroplasts confers resistance to plants against susceptible and Bt-resistant insects; *Proc. Natl. Acad. Sci. USA* **96** 1840–1845

Kowalchuk G A, Bruinsma M and van Veen J A 2003 Assessing responses of soil microorganisms to GM plants; *Trends Ecol. Evol.* **18** 403–410

Kramer V C, Morgan M K, Anderson A R, Hart H P, Warren G W *et al* 2001 Insecticidal toxins from *Photorhabdus luminescens* and nucleic acid sequences coding there for; *US Pat.* No. 6281413

Kramer K J, Morgan T D, Throne J E, Dowell F E, Bailey M and Howard J A 2000 Transgenic avidin maize is resistant to storage insect pests; *Nat. Biotechnol.* **18** 670–674

Lavigne C, Manach H, Guyard C and Gasquez J 1995 The cost of herbicide resistance in white-chicory: Ecological implications for its commercial release; *Theor. Appl. Genet.* **91** 1301–1308

Lee L, Saxena D and Stotzky G 2003 Activity of free and clay-bound insecticidal proteins from *Bacillus thuringiensis* subsp. israelensis against the mosquito *Culex pipiens; Appl. Environ. Microbiol.* **69** 4111–4115

Legere A 2005 Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus* L.) as a case study; *Pest Manag. Sci.* **61** 292–300

Liu Z C, Ye G Y and Hu C 2002 Effects of Bt transgenic rice on population dynamics of main non-target insect pests and dominant spider species in rice paddies; *Acta Phytophylacica Sin.* **29** 138–145

Lottmann J, Heuer H, Smalla K and Berg G 1999 Influence of transgenic T4-lysozyme-producing potato plants on potentially beneficial plant-associated bacteria; *FEMS Microbiol. Ecol.* **29** 365–377

Lu C M, Kato M and Kakihara F 2002 Destiny of a transgene escape from *Brassica napus* into *Brassica rapa; Theor. Appl. Genet.* **105** 78–84

Malone L A, Burgess E P J, Stefanovic D and Gatehouse H S 2000 Effects of four protease inhibitors on the survival of worker bumblebees, *Bombus terrestris* L.; *Apidologie* **31** 25–38

Malone L A, Tregidga E L, Todd J H, Burgess E P J, Philip B A, Markwick N P, Poulton J, Christeller J T, Lester M T and Gatehouse H S 2002 Effects of ingestion of a biotin-binding protein on adult and larval honey bees; *Apidologie* **33** 447–458

Marvier M 2002 Improving risk assessment for non target safety of transgenic crops; *Ecol. Appl.* **12** 1119–1124

Mason P, Braun L, Warwick S I, Zhu B and Stewart C N 2003 Transgenic Bt-producing *Brassica napus*: *Plutella xylostella* selection pressure and fitness of weedy relatives; *Environ. Biosafety Res.* **2** 263–276

McBride K E, Svab Z, Schaaf D J, Hogan P S, Stalker D M and Maliga P 1995 Amplification of a Chimeric Bacillus Gene in Chloroplasts Leads to an Extraordinary Level of an Insecticidal Protein in Tobacco; *Bio-Technology* **13** 362–365

McPartlan H C and Dale P J 1994 An assessment of gene-transfer by pollen from field-grown transgenic potatoes to nontransgenic potatoes and related species; *Transgenic Res.* **3** 216–225

Meagher T R, Belanger F C and Day P R 2003 Using empirical data to model transgene dispersal; *Philos. Trans. R. Soc. London B* **358** 115711–115762

Meier M S and Hilbeck A 2001 Influence of transgenic *Bacillus thuringiensis* cornfed prey on prey preference of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae); *Basic Appl. Ecol.* **2** 35–44

Meier P and Wackernagel W 2003 Monitoring the spread of recombinant DNA from field plots with transgenic sugar beet plants by PCR and natural transformation of *Pseudomonas stutzeri; Transgenic Res.* **12** 293–304

Mendelsohn M, Kough J, Vaituzis Z and Matthews K 2003 Are *Bt* crops safe?; *Nature Biotechnol.* **21** 1003–1009

Metz M and Futterer J 2002 Biodiversity (Communications arising): suspect evidence of transgenic contamination; *Nature* (*London*) **416** 600–601

Miller R M 1993 Nontarget and Ecological Effects of Transgenically Altered Disease Resistance in Crops–Possible Effects on the Mycorrhizal Symbiosis; *Mol. Ecol.* **2** 327–335

Motavalli P P, Kremer R J, Fanga M and Meansa N E 2004 Impact of Genetically Modified Crops and Their Management on Soil Microbially Mediated Plant Nutrient Transformations; *J. Environ. Qual.* **33** 816–824

Musser F R and Shelton A M 2003 Bt sweet corn and selective insecticides: impacts on pests and predators; *J. Econ. Entomol.* **96** 71–80

Nelson G C and Bullock D S 2003 Simulating a relative environmental effect of glyphosate-resistant soybeans; *Ecol. Econ.* **45** 189–202

Nielsen K M, van Elsas J D and Smalla K 2000 Transformation of *Acinetobacter* sp. strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants; *Appl. Environ. Microbiol.* **66** 1237–1242

Nielsen K M, Van Elsas J D and Smalla K 2001 Dynamics, horizontal transfer and selection of novel DNA in bacterial populations in the phytosphere of transgenic plants; *Ann. Microbiol.* **51** 79–94

Nuffield Bioethics Committee 2004 *The use of genetically modified crops in developing countries* (London: Nuffield Bioethics Committee)

O'Callaghan M, Glare T R, Burgess E P J and Malone L A 2005 Effect of plants genetically modified for insect resistance on non target organisms; *Annu. Rev. Entomol.* **50** 271–292

Pearce F 2002 The great Mexican maize scandal; *New Sci.* **174** 14–16

Picardnizou A L, Phamdelegue M H, Kerguelen V, Marilleau R, Olsen L, Grison R, Toppan A and Masson C 1995 Foraging Behavior of Honey-Bees (*Apis mellifera* L.) on Transgenic Oilseed Rape (*Brassica napus* L. Var Oleifera); *Transgenic Res.* **4** 270–276

Pilcher C D, Obrycki J J, Rice M E and Lewis L C 1997 Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn; *Environ. Entomol.* **26** 446–454

Pilcher C D, Rice M E, Higgins R A and Bowling R 2001 Pollen drift from *Bacillus thuringiensis* corn: Efficacy against European corn borer (Lepidoptera: Crambidae) in adjacent rows of non-Bt corn; *Environ. Entomol.* **30** 409–414

Ponsard S, Gutierrez A P and Mills N J 2002 Effect of Bt-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators; *Environ. Entomol.* **31** 1197–1205

Purrington C B and Bergelson J 1999 Exploring the physiological basis of costs of herbicide resistance in *Arabidopsis thaliana*; *Am. Nat.* **154** S82–S91

Puterka G J, Bocchetti C, Dang P, Bell R L and Scorza R 2002 Pear transformed with a lytic peptide gene for disease control affects nontarget organism, *pear psylla* (Homoptera: Psyllidae); *J. Econ. Entomol.* **95** 797–802

Quist D and Chapela I H 2001 Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico; *Nature* (*London*) **414** 541–543

Raps A, Kehr J, Gugerli P, Moar W J, Bigler F and Hilbeck A 2001 Immunological analysis of phloem sap of *Bacillus thurigiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab; *Mol. Ecol.* **10** 525–533

Reed G L, Jensen A S, Riebe J, Head G and Duan J J 2001 Transgenic Bt potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts; *Entomol. Exp. Appl.* **100** 89–100

Riddick E W, Dively G and Barbosa P 2000 Season-long abundance of generalist predators in transgenic versus non transgenic potato fields; *J. Entomol. Sci.* **35** 349–359

Romeis J, Babendreier D and Wackers F L 2003a Consumption of snowdrop lectin (*Galanthus nivalis* agglutinin) causes direct effects on adult parasitic wasps; *Oecologia* **134** 528–536

Romeis J, Battini M and Bigler F 2003b Transgenic wheat with enhanced fungal resistance causes no effects on *Folsomia candida* (Collembola: Isotomidae); *Pedobiologia* **47** 141–147

Romeis J, Dutton A and Bigler F 2004 *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae); *J. Insect. Physiol.* **50** 175–183

Saxena D, Flores S and Stotzky G 1999 Transgenic plants: insecticidal toxin in root exudates from Bt corn; *Nature* (*London*) **402** 480

Saxena D, Flores S and Stotzky G 2002 Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events; *Soil Biol. Biochem.* **34** 133–137

Saxena D, Stewart C N, Altosaar I, Shu Q and Stotzky G 2004 Larvicidal Cry proteins from *Bacillus thuringiensis* are released in root exudates of transgenic *B. thuringiensis* corn, potato, and rice but not of *B. thuringiensis* canola, cotton, and tobacco; *Plant Physiol. Biochem.* **42** 383–387

Saxena D and Stotzky G 2001a *Bacillus thuringiensis* (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria and fungi in soil; *Soil Biol. Biochem.* **33** 1225–1230

Saxena D and Stotzky G 2001b *Bt* corn has a higher lignin content than non-*Bt* corn.; *Am. J. Bot.* **88** 1704–1706

Schmalenberger A and Tebbe C C 2002 Bacterial community composition in the rhizosphere of a transgenic, herbicide-resistant maize (*Zea mays*) and comparison to its non-transgenic cultivar Bosphore; *FEMS Microbiol. Ecol.* **40** 29–37

Schmalenberger A and Tebbe C C 2003 Genetic profiling of noncultivated bacteria from the rhizospheres of sugar beet (*Beta vulgaris*) reveal field and annual variability but no effect of a transgenic herbicide resistance; *Can. J. Microbiol.* **49** 1–8

Schmitz G, Bartsch D and Pretscher P 2003 Selection of relevant non-target herbivores for monitoring the environmental effects of Bt maize pollen; *Environ. Biosafety* **2** 117–132

Schuler T H, Denholm I, Jouanin L, Clark S J, Clark A J and Poppy G M 2001 Population-scale laboratory studies of the effect of transgenic plants on nontarget insects; *Mol. Ecol.* **10** 1845–1853

Schuler T H, Potting R P J, Denholm I, Clark S J, Clark A J, Stewart C N and Poppy G M 2003 Tritrophic choice experiments with Bt plants, the diamondback moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae; Transgenic Res.* **12** 351–361

Setamou M, Bernal J S, Legaspi J C and Mirkov T E 2002 Effects of snowdrop lectin (*Galanthus nivalis* agglutinin) expressed in transgenic sugarcane on fitness of *Cotesia flavipes* (Hymenoptera: Braconidae), a parasitoid of the nontarget pest *Diatraea saccharalis* (Lepidoptera: Crambidae); *Ann. Entomol. Soc. Am.* **95** 75–83

Shelton A M, Zhao J Z and Roush R T 2002 Economic, ecological, food safety and social consequences of the deployment of bt transgenic plants; *Annu. Rev. Entomol.* **47** 845–881

Simonet P 2000 An evaluation of the possibility of transferring DNA from GM crops to soil bacteria; *OCL-Ol Corps Gras Lipides* **7** 320–323

Sims S R 1995 *Bacillus thuringiensis* var kurstaki CryIA(c) protein expressed in transgenic cotton: Effects on beneficial and other non-target insects; *Southw. Entomol.* **20** 493–500

Snow A A, Pilson D Rieseberg L H, Paulsen M J, Pleskac N, Reagon M R, Wolf D E and Selbo S M 2003 A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers; *Ecol. Appl.* **13** 279–286

Stoger E, Williams S, Christou P, Down R E and Gatehouse J A 1999 Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae; Mol. Breed* **5** 65–73

Stotzky G 2000 Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids; *J. Environ. Qual.* **29** 691–705

Stotzky G 2002 Release, persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis;* in *genetically engineered organisms: Assessing environmental and human health effects* (ed.) D K Letourneau and B E lpern Burrows (Boca Raton, FL: CRC Press) pp 187–222

Stewart C N, All J N, Raymer P L and Ramachandran S 1997 Increased fitness of transgenic insecticidal rapeseed under insect selection pressure; *Mol. Ecol.* **6** 773–779

Stewart C Neal Jr Halfhill M D and Warkwick S I 2003 Trans-

gene introgression from genetically modified crops to their wild relatives; *Nature Rev. Genet.* **4** 806–817

Tepfer D, Garcia-Gonzales R, Mansouri H, Seruga M, Message B, Leach F and Perica M C 2003 Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer; *Transgenic Res.* **12** 425–437

Tomov B W, Bernal J S and Vinson S B 2003 Impacts of transgenic sugarcane expressing GNA lectin on parasitism of Mexican rice borer by *Parallorhogas pyralophagus* (Marsh) (Hymenoptera: Braconidae); *Environ. Entomol.* **32** 866–872

Tschenn J, Losey J E, Jesse L H, Obrycki J J and Hufbauer R 2001 Effects of corn plants and corn pollen on monarch butterfly (Lepidoptera: Danaidae) oviposition behavior; *Environ. Entomol.* **30** 495–500

Turrini A, Sbrana C, Nuti M P, Pietrangeli B and Giovannetti M 2004 Development of a model system to assess the impact of genetically modified corn and aubergine plants on arbuscular mycorrhizal fungi; *Plant Soil.* **266** 69–75

United States Environmental Protection Agency 2000 *Bt plant-pesticides biopesticides registration action document* http://www.epa.gov.oscpmont/sap/2000/october/brad3 sment.pdf

Vacher C, Weis A E, Hermann D, Kossler T, Young C and Hochberg M E 2004 Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (*Brassica rapa*); *Theor. Appl. Genet.* **109** 806–814

Velkov V V, Sokolov M S and Medvinsky A B 2003a The Problems of the State Regulation of Production of Transgenic Plants, (Russ.); *Vestnik zashchity rastenii* (*Plant Protection News*) **3** 3–16

Velkov V V, Sokolov M S and Medvinsky A B 2003b Assessment of the Ecological Risks of Production of the Transgenic Insecticidal Plants, (Russ.); *Agrokhimiya* (*Agricultural Chemistry*) **2** 74–96

Wang X, Ding X, Gopalakrishnan B, Morgan T D, Johnson L *et al* 1996 Characterisation of a 46 kDa insect chitinase from transgenic tobacco; *Insect Biochem. Mol. Biol.* **10** 1055–1064

Wang W, Vinocur B and Altman A 2003 Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance; *Planta.* **218** 1–14

Watrud L S, Lee E H, Fairbrother A, Burdick C, Reichman J R, Bollman M, Storm M, King G and Van de Water P K 2004 Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker; *Proc. Natl. Acad. Sci. USA* **101** 14533–14538

Wei-xiang Wu, Qing-fuYe and Hang Min 2004 Effect of straws from Bt-transgenic rice on selected biological activities in water-flooded soil; *Eur. J . Soil Biol.* **40** 15–22

Widmer F, Seidler R J and Watrud L S 1996 Sensitive detection of transgenic plant marker gene persistence in soil microcosms; *Mol. Ecol.* **5** 603–613

Wisniewski J-P, Frangne P, Massonneau A and Dumas C 2002 Between myth and reality: genetically modified maize, an example of a sizeable scientific controversy; *Biochimie* **84** 1095–1103

Wold S, Burkness E C, Hutchinson W D and Venette R C 2001 In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin; *J. Entomol. Sci.* **36**177–186

Wolt J D, Peterson R K D, Bystrak P and Meade T 2003 A screening level approach for nontarget insect risk assessment: Transgenic Bt corn pollen and the monarch butterfly (Lepidoptera: Danaidae); *Environ. Entomol.* **32** 237–246

Wolfenbarger L L and Phifer P R 2000 Biotechnology and ecology – the ecological risks and benefits of genetically engineered plants; *Science* **290** 2088–2093

Wraight C L, Zangerl A R, Carroll M J and Berenbaum M R 2000 Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions; *Proc. Natl. Acad. Sci. USA* **97** 7700–7703

Wu K, Peng Y and Jia S 2003 What we have learnt on impacts of *Bt* cotton on non-target organisms in China; *AgBiotechNet* **5** ABN 112 1171

Wu K M and Guo Y Y 2005 The evolution of Cotton Pest Management practices in China; *Annu. Rev. Entomol.* **50** 31–52

Yu L, Berry R E and Croft B A 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

Zablotowicz R M and Reddy K N 2004 Impact of Glyphosate on the *Bradyrhizobium japonicum* Symbiosis with Glyphosate-Resistant Transgenic Soybean: A Minireview; *J. Environ. Qual.* **33** 825–831

Zangerl A R, McKenna D, Wraight C L, Carroll M, Ficarello P, Warner R and Berenbaum M R 2001 Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions; *Proc. Natl. Acad. Sci. USA* **98** 11908–11912

Zhu B, Lawrence J R, Warwick S I, Mason P, Braun L, Halfhill M D and Stewart C N Jr 2004 Inheritance of GFP-Bt transgenes from *Brassica napus* in backcrosses with three wild *B. rapa* accessions; *Environ. Biosafety Res.* **3** 45–54

Zwahlen C, Hilbeck A, Gugerli P and Nentwig W 2003a Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field; *Mol. Ecol.* **12** 765–775

Zwahlen C, Hilbeck A, Howald R and Nentwig W 2003b Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris; Mol. Ecol.* **12** 1077–1086

Zwahlen C, Nentwig W, Bigler F and Hilbeck A 2000 Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and the predator *Orius majusculus* (Heteroptera: Anthocoridae); *Environ. Entomol.* **29** 846–850

MS received 14 March 2005; accepted 18 May 2005

*e*Publication 23 August 2005

Corresponding editor: IMRAN SIDDIQI