

# Annotated Bibliography on Environmental and Ecological Impacts from Transgenic Plants II: Unintended Effects

Compiled by L. LaReesa Wolfenbarger

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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.

An environmental risk evaluation of a transgenic strawberry expressing a rice chitinase gene was carried out in a closed greenhouse, a semi-closed greenhouse, and an isolated field. The transgenic strawberry did not produce 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 green house. 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.

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.

An environmental concern regarding the cultivation of transgenic crop plants is their effect on non-target organisms. 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. We employed a laboratory larval rearing technique to test the impacts of such transgene products on honey bees. A serine proteinase inhibitor (Kunitz Soybean Trypsin Inhibitor, SBTI), that is a source of insect resistance in transgenic plants, 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. Our results suggest that the larval rearing technique can be used to monitor direct side-effects of transgene products on individual honey bee larvae.

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

Protease inhibitors expressed in transgenic plants can provide enhanced levels of resistance to important pest species. A sequential approach for testing the effects of protease inhibitor-expressing crops on nontarget herbivorous insects has been developed. The approach consists of five tiers. The first two tiers comprise the selection phase. In tier one, field surveys are used to characterise the nontarget invertebrate fauna of a crop. In tier 2, histochemical assays are 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) are used to evaluate the impact of the protease inhibitor-expressing plants on the selected nontarget species. In the final tier, field trials are 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 are described using potatoes expressing cystatins, a family of cysteine proteinase inhibitors, as an example. Although the plants have enhanced levels of resistance to potato cyst nematodes (PCN), *Globodera pallida* and *Globodera rostochiensis*, the results establish that they have negligible impact on the nontarget herbivorous insect, *Eupteryx aurata*.

Dewar, A. M., May, M. J., Woiwod, I. P., Haylock, L. A., Champion, G. T., Garner, B. H., Sands, R. J. N., Qi, A. M. and Pidgeon, J. D. 2003. A novel approach to the use of genetically modified herbicide tolerant crops for environmental benefit. *Proc R Soc Lond Ser B-Biol Sci* **270**: 335-340.

The proposed introduction of genetically modified herbicide tolerant (GMHT) crops, with claims of improved weed control, has prompted fears about possible environmental impacts of their widespread adoption, particularly on arable weeds, insects and associated farmland birds. In response to this, we have developed a novel weed-management system for GMHT sugar beet, based on band spraying, which exploits the flexibility offered by the broad-spectrum partner herbicides. Here, we show the results from two series of field experiments which, taken together, demonstrate that, by using this system, crops can be managed for enhanced weed and insect biomass without compromising yield, thus potentially offering food and shelter to farmland birds and other wildlife. These results could be applicable widely to other row crops, and indicate that creative use of GMHT technology could be a powerful tool for developing more sustainable farming systems in the future.

Dutton, A., Klein, H., Romeis, J. and Bigler, F. 2003. Prey-mediated effects of *Bacillus*

thuringiensis spray on the predator *Chrysoperla carnea* in maize. *Biol Control* **26**: 209-215.

*Bacillus thuringiensis* (Bt) bioinsecticides are generally considered safe to beneficial insects. However, negative effects of transgenic Bt-expressing maize on an important predator, *Chrysoperla carnea*, have previously been reported. Here, we make an ecological assessment of the effects of Dipel, one of the most widely used Bt-sprays in agriculture, on *C. carnea* larvae. Indirect effects due to a reduction of prey were tested by rearing three prey species of *C. carnea* (the aphid *Rhopalosiphum padi*, the spider mite *Tetranychus urticae*, and Lepidoptera larva *Spodoptera littoralis*) on either maize 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. The effects of Dipel on *C. carnea* larvae shown in this study, are comparable to earlier published results obtained with Bt maize. The ecological relevance of these results is discussed in comparison with the possible risks that the deployment of Bt-expressing maize plants pose to the predator *C. carnea*. (C) 2002 Elsevier Science (USA). All rights reserved.

Glawe, G. A., Zavala, J. A., Kessler, A., Van Dam, N. M. and Baldwin, I. T. 2003. Ecological costs and benefits correlated with trypsin protease inhibitor production in *Nicotiana attenuata*. *Ecology* **84**: 79-90.

Genotypes of the wild tobacco *Nicotiana attenuata* from different geographic regions in North America vary considerably in the level of constitutive and inducible trypsin protease inhibitors (TrypPIs), a potent direct defense, as well as in the production of herbivore-induced volatiles that function as indirect defense. Genotypes collected from Arizona were found to lack the ability to produce TrpPIs at a transcriptional level, had decreased volatile production, but exhibited nicotine and growth responses that were not distinguishable from genotypes collected in Utah. In field trials with naturally occurring herbivores and in lab experiments with *Manduca sexta* larvae, Arizona genotypes were damaged more and sustained greater herbivore growth than the Utah genotypes. When Arizona and Utah genotypes were grown in competition, Arizona genotypes produced significantly more seed capsules than the Utah neighbor did. Moreover, jasmonate elicitation, which dramatically increased TrypPI production in only the Utah genotypes, reduced lifetime fitness measures of the Utah genotypes more than of the Arizona genotypes, demonstrating that TrypPI production is correlated with a fitness cost. The loss of both a direct and an indirect defense suggests a functional linkage between these types of defense.

Nelson, G. C. and Bullock, D. S. 2003. Simulating a relative environmental effect of glyphosate-resistant soybeans. *Ecological Economics* **45**: 189-202.

Great controversy surrounds the potential environmental effects of genetically modified crops (GMOs). Of the GM crops currently in commercial use, glyphosate-resistant (GR) soybeans have probably received the most attention from both proponents and opponents of GMOs. GR 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. Measuring and valuing these effects completely is not possible today. Environmental indicators are attempts to find more easily measured proxies for the external effects. We present an environmental indicator based on a standardized, well-known acute mammalian toxicity measure, the LD50 dose for rats. We use this indicator to compare an environmental effect of the use of GR genetically modified soybeans to the use of non-modified soybeans for over 1400 US Midwest farms. This indicator is superior to previous indicators used to assess environmental effects of GR soybeans that rely on adding up volumes of different herbicides. The indicator allows for consistent aggregation of a specific environmental effect across many different pesticides. Furthermore, the methodology can be used to compare environmental effects for many other types of technology choices since values are mandated for all pesticides. Our simulation results suggest that GR soybean seed technology is more environmentally friendly than non-GR 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. (C) 2003 Elsevier Science B.V. All rights reserved.

Romeis, J., Battini, M. and Bigler, F. 2003. Transgenic wheat with enhanced fungal resistance

causes no effects on *Folsomia candida* (Collembola : Isotomidae). *Pedobiologia* **47**: 141-147.

The potential environmental impact of genetically modified (GM) plants is a major concern arising from the use of these novel crops. Therefore an ecological risk assessment should be done prior to the wide scale use of a GM crop. Soil microarthropods are a group of non-target organisms that should be considered as part of this risk assessment due to their importance for the decomposition process of organic matter and due to their intense exposure to crop residues in the soil. In the study described here, two different transgenic wheat varieties were used that express 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 show an enhanced resistance against stinking smut, *Tilletia tritici*. We describe different laboratory bioassays and a glasshouse study where we tested effects of the two transgenic KP4 wheat varieties on the Collembola *Folsomia candida*. Feeding bioassays in which dried root material from transgenic and non-transgenic wheat plants was fed to individual *F. candida* revealed no effect of transformation or wheat variety on any of the life-history parameters evaluated. The comparison to an optimal food source (yeast) showed that certain parameters, i.e. insect development, egg cluster size and insect weight, are very sensitive parameters to detect differences in food quality. Other parameters including egg development and egg viability, revealed no difference between plant material and yeast fed *F. candida*. The glasshouse study showed that population development of *F. candida* did not differ between pots in which transgenic or non-transgenic plants of the two varieties were grown. However, a significant variety effect was detected.

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. *Canadian Journal of Microbiology* **49**: 1-8.

In this field study, we compared the bacterial communities inhabiting the rhizosphere of a transgenic, herbicide-resistant sugar beet (*Beta vulgaris*) cultivar with those of its nonengineered counterpart, using a genetic profiling technique based on PCR amplifications of partial 16S rRNA gene sequences and single-strand conformation polymorphism (SSCP). As a control for the plasticity of the bacterial community, we also analyzed 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. PCR was carried out with primers that hybridized to evolutionarily conserved regions flanking variable regions 4 and 5 of the 16S rRNA gene. SSCP patterns of these PCR products were composed of approximately 50 distinguishable bands, as detected by silver staining of the gels after electrophoresis. 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.

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.

Parasitoids are important natural enemies of many pest species and are used extensively in biological and integrated control programmes. Crop plants transformed to express toxin genes derived from *Bacillus thuringiensis* (Bt) provide high levels of resistance to certain pest species, which is likely to have consequent effects on parasitoids specialising on such pests. A better understanding of the interaction between transgenic plants, pests and parasitoids is important to limit disruption of biological control and to provide background knowledge essential for implementing measures for the conservation of parasitoid populations. It is also essential for investigations into the potential role of parasitoids in delaying the build-up of Bt-resistant pest populations. 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 toxins 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 presented in this paper 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. The implications for

integrated pest management and the evolution of resistance to Bt in *P. xylostella* are discussed.

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.

Quantitative risk assessment affords an objective approach for assessing ecological risk from crops produced using biotechnology. Ecological risk assessment for plant-incorporated insecticidal proteins necessitates consideration of risks to nontarget insects when species-specific hazard information may be lacking. Screening-level risk assessment methods afford a means by which risks to species of concern may be evaluated conservatively using exposure estimates, host-range information, and a probabilistic estimate of toxicity to sensitive species. This approach was applied to the special case of Bt corn pollen risk, to monarch butterfly, *Danaus 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 based on readily available literature 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 effect (LC50.) was used to estimate monarch butterfly sensitivity to Bt corn pollen expressing Cry1A (b) protein, the risk of lethality to individual larvae was negligible at > 1 in from the edge of source corn fields. Subsequent field measurements of pollen distribution, interception by milkweed, and especially effects determinations for monarch butterfly larvae exposed to Cry1A (b) toxin indicate that the screening-level approach was effective in focusing the scope of the problem to exposure from high-expressing Cry1A(b) events occurring within source cornfields or at the near-field edge. Screening level risk assessment conservatively identifies the scope of concern and the uncertainties that need clarification so that subsequent research can be appropriately focused.

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.

Five transgenic rice lines, each containing an insecticidal toxin gene from *Bacillus thuringiensis* (Bt) under control of a different promoter, 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 rice lines with the CaMV 35S and actin promoters. *Nilaparvata lugens* produced greater volumes of acidic honeydew (derived from xylem feeding) on all five Bt rice lines than on non-transgenic control lines. The amount of honeydew derived from phloem feeding did not differ 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). There were no differences between *C. lividipennis* reared on *N. lugens* nymphs from Bt and control lines, in any of the three fitness parameters examined (survival to the adult stage and male and female developmental time). Our results indicate that *N. lugens* and its natural enemies will be exposed to Bt toxins from rice lines transformed with some Bt gene constructs, but that this exposure might not affect *N. lugens* and *C. lividipennis* fitness.

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.

Transgenic Bt maize cultivars are widely used in U.S. agriculture. Control of target pests by these cultivars is not complete, and non-target pests are usually tolerant of the toxic activity of these cultivars. Sublethally intoxicated pest individuals may become hosts for parasitoids, but their quality as hosts may be affected as a result of intoxication. This study addresses 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 maize 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 d shorter in females, and developmental times were approximately 2 d longer in both males and females when *P. pyralophagus* developed on hosts fed Bt maize tissue relative to hosts fed non-Bt maize tissue. Moreover, parasitoid brood size was positively correlated with host size when hosts were fed non-Bt maize tissue, while this relationship was absent when hosts were fed Bt maize tissue. However, feeding on Bt maize 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 maize tissue. Results are

discussed in relation to the importance of sublethal effects of Bt maize cultivars on parasitoids and potential impacts on naturally-occurring biological control.

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.

The degree of abundance and diversity of springtails (order Collembola) often indicates the extent of disturbance by various agricultural practices. We examined how transgenic herbicide-tolerant 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), we tested three weed management systems: (1) targeted application of specific herbicides to the corresponding tolerant transgenic varieties; (2) conventional pre- and postemergence herbicide applications; and (3) 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. We attributed the observed treatment effect differences 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. Our results overall suggested no deleterious short-term effects of transgenic soybean targeted weed-management systems on abundance of the springtail species examined.

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.

To investigate the potential non-target impacts of transgenic pest-resistant plants, prey-mediated impacts of a protease inhibitor (PI) on the predatory carabid, *Nebria brevicollis*, were investigated. The PI used was aprotinin, a serine PI of mammalian origin with insecticidal properties when incorporated in artificial diet or expressed in transgenic plants. Field-collected *N. brevicollis* adults, kept at 23 degreesC, 16:8 L:D, 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 mug 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 we found no consistent gender-based differences in response to PI-prey. 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. (C) 2002 Elsevier Science Ltd. All rights reserved.

Coviella, C. E., Stipanovic, R. D. and Trumble, J. T. 2002. Plant allocation to defensive compounds: interactions between elevated CO<sub>2</sub> and nitrogen in transgenic cotton plants. *J Exp Bot* **53**: 323-331.

Plant allocation to defensive compounds in response to growth in elevated atmospheric CO<sub>2</sub> in combination with two levels of nitrogen was examined. The aim was to discover if allocation patterns of transgenic plants containing genes for defensive chemicals which had not evolved in the species would respond as predicted by the Carbon Nutrient Balance (CNB) hypothesis. Cotton plants (*Gossypium hirsutum* L.) were sown inside 12 environmental chambers. Six of them were maintained at an elevated CO<sub>2</sub> level of 900 mumol mol<sup>-1</sup> and the other six at the current level of similar to 370 mumol mol<sup>-1</sup>. Half the plants in each chamber were from a transgenic line producing *Bacillus thuringiensis* (Bt) toxin and the others were from a near isogenic line without the Bt gene. The allocation to total phenolics, condensed tannins, and gossypol and related terpenoid aldehydes was measured. All the treatments were bioassayed against a non-target insect herbivore found on cotton, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Plants had lower N concentrations and higher C:N ratios when grown in elevated CO<sub>2</sub>. Carbon defensive compounds increased in elevated CO<sub>2</sub>, low N availability or both. The increase in these compounds in elevated CO<sub>2</sub> and low N, adversely affected growth and survival of *S. exigua*. The production of the nitrogen-based toxin was affected by an interaction between CO<sub>2</sub> and N; elevated CO<sub>2</sub> decreased N

allocation to Bt, but the reduction was largely alleviated by the addition of nitrogen. The CNB hypothesis accurately predicted only some of the results, and may require revision. These data indicate that for the future expected elevated CO<sub>2</sub> concentrations, plant allocation to defensive compounds will be affected enough to impact plant- herbivore interactions.

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

1. Plant-parasitic nematodes are important pests of agriculture and transgenic plants with potential for nematode control are currently being developed. The expression of cysteine proteinase inhibitors (cystatins) in potato confers partial resistance to potato-cyst nematode (PCN). Here, we used field studies to test for effects of cystatin-expressing potato on non-target soil organisms. 2. 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. 3. 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 18:2omega6 in late season, suggesting a suppression of fungal growth. 4. 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. 5. The observed changes in soil microbial community structure did not result in changes in the rate of leaf litter decomposition. 6. The transgenic lines had no significant effect on the abundance of soil microarthropods or free-living nematodes. 7. The study is the first stage of a risk assessment of the impact of transgenic nematode resistance on non-target soil organisms. It has highlighted the importance of including currently used management options when studying the effect of transgenic plants on non-target organisms. Both nematicide use 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, S. E., Wright, C. and Atkinson, H. J. 2002. Transgenic potatoes with enhanced levels of nematode resistance do not have altered susceptibility to nontarget aphids. *Mol Ecol* **11**: 821-827.

Cysteine proteinase inhibitors (cystatins) confer resistance to plant-parasitic nematodes when expressed in transgenic plants. 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 Desiree plants. The effect of other nematode management options on aphid numbers was also studied. A conventionally bred cultivar, with partial nematode resistance, supported higher populations of aphids than the transgenic lines at the beginning of the sampling period. Peak aphid densities on the untreated control and untreated transgenic lines were 7 and 5.2 aphids per plant. Aldicarb, commonly used to control nematodes on potatoes, reduced the value to less than 0.2 aphids per plant. The results demonstrate that levels of expression in the plant tissue actually consumed are important in determining the risk of cystatins to nontarget invertebrates. The study also highlights the importance of including currently used management options in any assessment of the impact of transgenic plants on nontarget organisms.

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.

A transgenic corn event (MON 863) has been recently developed by Monsanto Company for control of corn rootworms, *Diabrotica* spp. (Coleoptera: Chrysomelidae). This transgenic corn event expresses the cry3Bb1 gene derived from *Bacillus thuringiensis* (Berliner), which encodes the insecticidal Cry3Bb1 protein for corn rootworm control. A continuous feeding study was conducted in the laboratory to evaluate the dietary effect of MON 863 pollen expressing the Cry3Bb1 protein on the survival, larval development, and reproductive capacity of the nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). First instar *C. maculata* (less than 24 h old) and newly emerging adults (less than 72 h old) were fed individually on a diet mixture containing 50% of MON 863 pollen, non-transgenic

(control) corn pollen, bee pollen (a component of normal rearing diet), or potassium arsenate- treated control corn pollen. In the larval tests, 96.7%, 90.0%, and 93.3% of *C. maculata* larvae successfully pupated and then emerged as adults when fed on MON 863 pollen, non-transgenic corn pollen, and bee pollen (normal rearing) diets, respectively. Among the larvae completing their development, there were no significant differences in the developmental time to pupation and adult emergence among the transgenic corn pollen, non-transgenic corn pollen, and bee pollen diet treatments. All larvae fed on arsenate treated corn pollen diet died as larvae. For tests with adults, 83.3%, 80.0%, and 100% of adult *C. maculata* survived for the 30 days of the test period when reared on diets containing 50% of MON 863 pollen, non-transgenic corn pollen, and bee pollen respectively. While the adult survival rate on MON 863 pollen diet was significantly less than that on the bee pollen diet, there was no significant difference between the MON 863 and non-transgenic corn pollen treatments. During the period of adult testing, an average of 77, 80, and 89 eggs per female were laid by females fed on the MON 863 pollen, control corn pollen, and bee pollen, respectively; no significant differences were detected in the number of eggs laid among these treatments. These results demonstrate that when offered at 50% by weight of the dietary component, transgenic corn (MON 863) 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. Relevance of these findings to ecological impacts of transgenic Bt crops on non-target beneficial insects is discussed.

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.

1. *Chrysoperla carnea* is an important predatory insect in maize. To assess the ecological effects of Bt-maize, expressing the Cry1Ab protein, on larvae of this predator, the following factors were examined: (1) the performance of three prey herbivores (*Rhopalosiphum padi*, *Tetranychus urticae*, and *Spodoptera littoralis*) on transgenic Bt and non-transgenic maize plants; (2) the intake of the Cry1Ab toxin by the three herbivores; and (3) the effects on *C. carnea* when fed each of the prey species. 2. The intrinsic rate of natural increase ( $r(m)$ ) was used as a measure of performance for *R. padi* and *T. urticae*. No difference in this parameter was observed between herbivores reared on Bt or non-transgenic plants. In contrast, a higher mortality rate and a delay in development were observed in *S. littoralis* larvae when fed Bt-maize compared with those fed the control maize plants. 3. 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*. 4. 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-maize. 5. A combined interaction of poor prey quality and Cry1Ab toxin may account for the negative effects observed on *C. carnea* when fed *S. littoralis*. The relevance of these findings to the ecological risks of Bt-maize on *C. carnea* is discussed.

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

Transgenic strawberry lines constitutively 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.). Data are presented on plant performance over three seasons (years). Field results for two years confirmed previous glasshouse findings and demonstrate protection in terms of attack by vine weevil larvae on all the transgenic lines. Over three years, two 'Melody' lines and three 'Symphony' lines continued to demonstrate significantly improved plant growth compared with control lines. The genotype into which CpTi was inserted had a significant effect on performance. 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 to be significantly affected by the CpTi transgenic lines.

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.

Soil samples were collected from within and outside six fields where insect-resistant transgenic cotton (Bollgard) encoding the *Bacillus thuringiensis* Berliner (Bt) subsp. *kurstaki* cryIAc gene had been grown and subsequently incorporated into soil by postharvest tillage for 3-6 consecutive years. The level of



CryI<sub>Ac</sub> protein in these samples (collected 3 mo after the last season's tillage) was evaluated using both enzyme-linked immunosorbent assays (ELISA) and bioassays with a susceptible insect species, *Heliothis virescens* (F.), the tobacco budworm. Both methods revealed that no detectable CryI<sub>Ac</sub> protein was present in any of the soil samples collected from within or outside the Bollgard fields. Based on the results from reference standards, the limit of detection for the ELISA was 3.68 ng of extractable protein per gram of soil, and that of the bioassay (measured by EC<sub>50</sub>) was 8 ng of biologically active protein per gram of soil. Together, these findings demonstrate that the amount of CryI<sub>Ac</sub> protein accumulated as a result of continuous use of transgenic 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.

Hu, G. and St Leger, J. 2002. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. *Appl Environ Microbiol* **68**: 6383-6387.

In the summer of 2000, we released genetically altered insect- pathogenic fungi onto a plot of cabbages at a field site on the Upper Marlboro Research Station, Md. The transformed derivatives of *Metarhizium anisopliae* ARSEF 1080, designated GPMa and GMa, carried the *Aequorea victoria* green fluorescent protein (gfp) gene alone (GMa) or with additional protease genes (Pr1) (GPMa). The study (i) confirmed the utility of GFP for monitoring pathogen strains in field populations over time, (ii) demonstrated little dissemination of transgenic strains and produced no evidence of transmission by nontarget insects, (iii) found that recombinant fungi were genetically stable over 1 year under field conditions, and (iv) determined that deployment of the transgenic strains did not depress the culturable indigenous fungal microflora. The major point of the study was to monitor the fate (survivorship) of transformants under field conditions. In nonrhizosphere soil, the amount of GMa decreased from 10(5) propagules/g at depths of 0 to 2 cm to 10(3) propagules/g after several months. However, the densities of GMa remained at 105 propagules/g in the inner rhizosphere, demonstrating that rhizospheric soils are a potential reservoir for *M. anisopliae*. These results place a sharp focus on the biology of the soil/root interphase as a site where plants, insects, and pathogens interact to determine fungal biocontrol efficacy, cycling, and survival. However, the rhizospheric effect was less marked for GPMa, and overall it showed reduced persistence in soils than did GMa.

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.

Many non-target lepidopteran species are potentially at risk from transgenic corn tissues (i.e., pollen and anthers) expressing a *Bacillus thuringiensis* Berliner derived toxin, however, risk assessment data is currently lacking for most species. A laboratory experiment examining the effect of transgenic Bt corn tissue on *Euchatias egle* Drury (Lepidoptera: Arctiidae) showed no larval mortality following a 48-hour exposure to Bt corn tissues on its food source, *Asclepias syriaca* (Asclepiadaceae). Two Bt corn events were used; 176, which expressed 1.60 µg Bt/g of pollen and anther tissue; and Bt11, which expressed 0.39 µg Bt/g pollen and anther tissue. 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 appears that *E. egle* will not be adversely affected by the wide-scale planting of Bt corn.

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

Transgenic *Brassica napus* can be easily crossed with wild *Brassica rapa*. The spread of the transgene to wild species has aroused the general concern about its effect on ecological and agricultural systems. This paper 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 backcross 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<sub>1</sub> generation, and decreased by 50% with the advance of each backcross generation; that of a C- chromosome gene was around 39.9% in BC<sub>1</sub>, 7.7% in BC<sub>2</sub>, 1.2% in BC<sub>3</sub> and 0.1% in the BC<sub>4</sub> 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. The effect of genetic drift and intergenomic exchange on gene transmission rate was discussed. It is suggested that the transgene integrated on a C-chromosome (or better on a cytoplasm genome) is safer than that on an A-chromosome. The 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.

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.

The insecticidal properties of biotin-binding proteins (BBPs) have recently been exploited in transgenic plants. As BBPs have a broad spectrum of insect toxicity, their potential impacts on non-target insects such as honey bees need to be assessed. In this study, 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.

Marvier, M. 2002. Improving risk assessment for nontarget safety of transgenic crops. *Ecol Appl* **12**: 1119-1124.

In many countries, government regulations require environmental risk assessment prior to commercial sale and widespread planting of transgenic crops. Here I evaluate the design and statistical rigor of experiments used by industry to assess the safety of transgenic plants for nontarget organisms, as required under U.S. regulations. This review reveals that a few simple improvements in experimental design could greatly increase the rigor and information content of studies required under current regulations. For example, although most experiments were conducted for 1-4 wk, some of the tested species can live a year or more and could experience much longer periods of exposure. Moreover, the number of replicates used in these studies was generally quite small (usually 2-6 replicates per treatment), resulting in experiments that had little chance of detecting real effects. Clearly, sample sizes should be bolstered, and nonsignificant results should be accompanied by an analysis of statistical power. In addition, information readily available over the Internet is insufficient for a quantitative assessment of a transgenic crop's safety. Improved access to information regarding the details of risk assessment studies could greatly increase the public's ability to evaluate industry's claims of safety.

Ohlfest, J. R., Jesse, L. C. H., Jurenka, R. and Obrycki, J. J. 2002. Stability of insecticidal CryIAb protein in transgenic Bt corn pollen exposed to UV irradiation. *J Kans Entomol Soc* **75**: 48-51.

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.

The biology and behavior of pear psylla, *Cacopsylla pyricola* Foerster, on a transgenic clone of 'Bartlett' pear, *Pyrus communis* L., containing a synthetic antimicrobial gene, D5C1, was compared with that of a nontransgenic parental clone 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 original transformation was to enhance pear resistance to the bacterial disease fireblight caused by *Erwinia amylovora* (Burr.) Winslow et al. The biology and behavior of pear psylla on a transgenic clone were compared with a nontransgenic parental pear clone in short- (less than or equal to 7-d) and long-term (32-d) 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 clone. Our results suggest that pear psylla biology and behavior are initially enhanced on this transgenic pear clone. However, chronic exposure of psylla populations to transformed pear plants that express the nptII marker and lytic peptide genes had detrimental effects on pear psylla reproductive biology. Field studies would be required to determine the specific effects of each gene on pear psylla biology and behavior and whether these effects would be expressed under natural conditions. The four-fold reduction in psylla population levels that resulted on this disease resistant transgenic pear line would be an added benefit to a pear integrated pest management (IPM) program. Overall, this study demonstrates that genetically altering plants to control one particular organism can have unintentional yet beneficial effects against other nontarget pest organisms in agricultural crops.

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.

Bacterial communities in rhizospheres of transgenic maize (*Zea mays*, with the *pat*-gene conferring resistance to the herbicide glufosinate; syn. L-phosphinothricin) were compared to its isogenic, non-transgenic cultivar. Total DNA was extracted from bacterial cell consortia collected from rhizospheres of plants grown in an agricultural field. With the use of three different primer pairs binding to evolutionarily conserved regions of the bacterial 16S rRNA gene, partial sequences were amplified by polymerase chain reaction (PCR). The PCR products were subjected to single-strand conformation polymorphism (SSCP) to generate genetic profiles which corresponded to the diversity of the amplified sequences. Genetic profiles of rhizospheres consisted of 40-60 distinguishable bands depending on the chosen 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 profiles as investigated with digital image analysis. 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 maize plants collected 35 and 70 days after sowing. Sequencing of the dominant 30 products from one typical SSCP profile generated from transgenic maize 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-Verrucomicrobium, Planctomyces, Holophaga and to Gram-positive bacteria with a high G+C DNA content. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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.

*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 the snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) was developed 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: (1) artificial diet containing transgenic sugarcane tissue or (2) 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 females, and egg to adult developmental periods. The negative effects of transgenic sugarcane on *C. flavipes* detected in this study are 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. Results are discussed in relation to potential impacts of the transgenic cultivar on biological control of sugarcane borer by *C. flavipes*.

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.

The aim of model GENESYS is to rank cropping systems according to their risk of gene escape from genetically modified, herbicide tolerant winter oilseed rape cultivars to rapeseed volunteers. The model integrates the effects of crop succession and crop management at the level of a region. The first part of the model presented in this paper describes the temporal evolution of rapeseed 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. The extension of the temporal sub-model to include the genetic evolution of rapeseed volunteers and the spatial dimension is presented in a second paper [Colbach, N., Clermont-Dauphin, C., Meynard, J.M., 2001. *Agric. Ecosyst. Environ.*]. (C) 2001 Elsevier science B.V. All rights reserved.

Couty, A., de la Vina, G., Clark, S. J., Kaiser, L., Pham-Delegue, M. H. and Poppy, G. M. 2001. 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.

Snowdrop lectin (*Galanthus nivalis* agglutinin, GNA), has been shown to confer partial resistance to two potato aphids *Myzus persicae* and *Aulacorthum solani*, when incorporated in artificial diet and/or expressed in transgenic potato. 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. The amounts of GNA present in aphid and parasitoid tissues were estimated by western blotting. Results suggest 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 highlights the need to determine the exact "causes and effects" when assessing the ecological impact of transgenic plants on non-target beneficial insects. Such bioassays form the basis of a tiered risk assessment moving from laboratory studies assessing individuals towards field-scale experiments assessing populations. (C) 2001 Elsevier Science Ltd. All rights reserved.

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.

The equilibrium adsorption and binding of the active toxin from *Bacillus thuringiensis* subsp. *kurstaki* on complexes of montmorillonite-humic acids-Al hydroxypolymers, as well as the biodegradation and the insecticidal activity of the bound toxin, were studied. Seventy percent 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<sub>2</sub>O 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 toxin 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. The results of these studies indicate that the release of transgenic plants and microorganisms expressing truncated genes that encode active insecticidal toxins from *B. thuringiensis* could result in the accumulation of these toxins in soil as a consequence of binding on surface-active soil particles. 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. <(c)> 2001 Elsevier Science Ltd. All rights reserved.

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.

Chloroplast genetic engineering offers several advantages over nuclear transformation including high levels of gene expression and gene containment. However, a consequence of placing a transgene in the chloroplast genome is that the antibiotic resistance genes used as selectable markers are highly amplified. Engineering genetically modified (GM) crops without: the use of antibiotic resistance genes should eliminate the potential risk of their transfer to the environment or gut microbes. Therefore, the betaine aldehyde dehydrogenase (BADH) gene from spinach was used in this study as a selectable marker. The selection process involves conversion of toxic betaine aldehyde (BA) by the chloroplast BADH enzyme to non- toxic glycine betaine, which also serves as an osmoprotectant. Chloroplast transformation efficiency was 25-fold higher in BA selection than with spectinomycin. In addition, rapid regeneration was obtained. Transgenic shoots appeared within 12 days in 80% of leaf disks (up to 23 shoots per disk) under BA selection compared to 45 days in 15% of disks (1 or 2 shoots per disk) under spectinomycin selection. Southern blots confirmed stable integration of foreign genes into all of the chloroplast genomes (similar to

10,000 copies per cell) resulting in homoplasmy. Transgenic tobacco plants showed 15- to 18-fold higher BADH activity at different developmental stages than untransformed controls. Transgenic plants were morphologically indistinguishable from untransformed plants and the introduced trait was inherited stably in the subsequent generation. This is the first report of genetic engineering of the higher plant chloroplast genome without the use of antibiotic selection. The use of naturally occurring genes in spinach for selection, in addition to gene containment, should ease public concerns regarding GM crops.

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

Genetically modified plants are widely grown predominantly in North America and to a lesser extent in Australia, Argentina and China but their regions of production are expected to spread soon beyond these limited areas also reaching Europe where great controversy over the application of gene technology in agriculture persists. Currently, several cultivars of eight major crop plants are commercially available including canola, corn, cotton, potato, soybean, sugar beet, tobacco and tomato, but many more plants with new and combined multiple traits are close to registration. While currently agronomic traits (herbicide resistance, insect resistance) dominate, traits conferring "quality" traits (altered oil compositions, protein and starch contents) will begin to dominate within the next years. However, economically the most promising future lies in the development and marketing of crop plants expressing pharmaceutical or "nutraceuticals" (functional foods), and plants that express a number of different genes. From this it is clear that future agricultural and, ultimately, also natural ecosystems will be challenged by the large-scale introduction of 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. In times of severe global decline of biodiversity, pro-active precaution is necessary and careful consideration of the likely expected effects of transgenic plants on biodiversity of plants and insects is mandatory. In this paper possible implications of non-target effects for insect and plant biodiversity are discussed and a case example of such non-target effects is presented. In a multiple year research project, tritrophic and bitrophic effects of transgenic corn, expressing the gene from *Bacillus thuringiensis* (Bt-corn) that codes for the high expression of an insecticidal toxin (Cry1Ab), on the natural enemy species, *Chrysoperla carnea* (the green lacewing), was investigated. In these laboratory trials, we found prey-mediated effects of transgenic Bt-corn causing significantly higher mortality of *C. carnea* larvae. In further laboratory trials, we confirmed 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 *Spodoptera littoralis* fed transgenic Bt-corn and *S. littoralis* fed non-transgenic corn, larger instars showed a significant preference for *S. littoralis* fed non-transgenic corn while this was not the case when the choice was between Bt- and isogenic corn fed aphids. Field implications of these findings could be multifold but will be difficult to assess because they interfere in very intricate ways with complex ecosystem processes that we still know only very little about. The future challenge in pest management will be to explore how transgenic plants can be incorporated as safe and effective components of IPM systems and what gene technology can contribute to the needs of a modern sustainable agriculture that avoids or reduces adverse impacts on biodiversity? For mainly economically motivated resistance management purposes, constitutive high expression of Bt-toxins in transgenic plants is promoted seeking to kill almost 100% of all susceptible (and if possible heterozygote resistant) target pest insects. However, for pest management this is usually not necessary. Control at or below an established economic injury level is sufficient for most pests and cropping systems. It is proposed that partially or moderately resistant plants expressing quantitative rather than single gene traits and affecting the target pest sub-lethally may provide a more meaningful contribution of agricultural biotechnology to modern sustainable agriculture. Some examples of such plants produced through conventional breeding are presented. Non-target effects may be less severe allowing for better incorporation of these plants into IPM or biological control programs using multiple control strategies, thereby, also reducing selection pressure for pest resistance development.

Morse, R. J., Yamamoto, T. and Stroud, R. M. 2001. Structure of Cry2Aa suggests an unexpected receptor binding epitope. *Structure* 9: 409-417.

Background: Genetically modified (GM) crops that express insecticidal protein toxins are an integral part of modern agriculture. Proteins produced by *Bacillus thuringiensis* (Bt) during sporulation mediate the pathogenicity of Bt toward a spectrum of insect larvae whose breadth depends upon the Bt strain. These transmembrane channel-forming toxins are stored in Bt as crystalline inclusions called Cry proteins. These proteins are the active agents used in the majority of biorational pesticides and insect-resistant transgenic crops. Though Bt toxins are promising as a crop protection alternative and are ecologically friendlier than synthetic organic pesticides, resistance to Bt toxins by insects is recognized as a potential limitation to their application. Results: We have determined the 2.2 Angstrom crystal structure of the Cry2Aa protoxin by

multiple isomorphous replacement. This is the first crystal structure of a Cry toxin specific to Diptera (mosquitoes and flies) and the first structure of a Cry toxin with high activity against larvae from two insect orders, Lepidoptera (moths and butterflies) and Diptera. Cry2Aa also provides the first structure of the proregion of a Cry toxin that is cleaved to generate the membrane-active toxin in the larval gut. Conclusions: The crystal structure of Cry2Aa reported here, together with chimeric-scanning and domain-swapping mutagenesis, defines the putative receptor binding epitope on the toxin and so may allow for alteration of specificity to combat resistance or to minimize collateral effects on nontarget species. The putative receptor binding epitope of Cry2Aa identified in this study differs from that inferred from previous structural studies of other Cry toxins.

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.

Horizontal gene transfer has played an important role in the evolution of bacterial genomes. In this review, we outline the factors influencing bacterial utilization of horizontally transferred DNA with reference to the capture of plant transgenes in the phytosphere; including soil, rhizosphere, phyllosphere and the digestive tracts of soil mesofauna invertebrates. Moreover, the release of transgenic plant DNA in the phytosphere and the transfer of bacterial genes in plant environments by natural transformation is reviewed. Some characteristics of transgenes in transplastomic plants that may increase their likelihood of horizontal gene transfer are identified. Finally, new avenues for future studies with special reference to the elucidation of possible impacts of plant transgenes on the bacterial gene pool are suggested.

Pessel, F. D., Lecomte, J., Emeriau, V., Krouti, M., Messean, A. and Gouyon, P. H. 2001. Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theor Appl Genet* **102**: 841-846.

It is widely assumed that most cultivated plants cannot persist in natural or semi-natural habitats. Most people thus assume that the plants growing outside of fields (in particular oilseed rape along roadsides) find their origins in the current or previous year's cultivation of that crop. One consequence of this assumption is that the identity of plants growing on road verges is thought to reflect one of the cultivars currently or recently cultivated, while another consequence is the widespread belief that transgenic plants can be simply managed and controlled by stopping their cultivation. Our work shows that this assumption is false. We identify relict plants of a now unmarketable cultivar type of oilseed rape which have persisted in a semi-natural habitat (road verges) for at least 8 years according to farmer surveys in the studied area. More generally, we confirm that the dynamics of the feral oilseed rape plants of road verges is more complex than those resulting from spillage from agricultural machines or from neighbouring arable fields cultivated the previous year. Within the scope of transgenic oilseed rape cultivation, these results suggest that more studies on the dynamics of feral oilseed rape are needed in order to assess more precisely the risks of its invasiveness and its potential impact on genetic pollution between GM fields and non-GM fields.

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.

Commercial non-*Bacillus thuringiensis* (Bt) corn was planted adjacent to Bt corn to determine the effects of Bt corn pollen falling on non-Bt plants for control of European corn borer larvae, *Ostrinia 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, we counted European corn borer larvae and larval tunnels in the stalk and ear shank. There were no significant differences in European corn borer 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 reflect a typical field situation. Our results suggest that Bt pollen has minimal or no control of European corn borer larvae in adjacent rows of non-Bt corn under natural conditions. Bt pollen drifting onto adjacent non-Bt plants should not increase the risks related to resistance management.

Quist, D. and Chapela, I. H. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* **414**: 541-543.

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. Direct effects on non-target species (1,2), and the possibility of unintentionally transferring traits of ecological relevance onto landraces and wild relatives have also been

sources of concern(3,4). The degree of genetic connectivity between industrial crops and their progenitors in landraces and wild relatives is a principal determinant of the evolutionary history of crops and agroecosystems throughout the world(5,6). Recent introductions of transgenic DNA constructs into agricultural fields provide unique markers to measure such connectivity. For these reasons, the detection of transgenic DNA in crop landraces is of critical importance. Here we report the presence of introgressed transgenic DNA constructs in native maize landraces grown in remote mountains in Oaxaca, Mexico, part of the Mesoamerican centre of origin and diversification of this crop(7-9).

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

Phloem sap of transgenic *Bacillus thuringiensis* (Bt) corn expressing a truncated form of the B. thuringiensis delta - endotoxin Cry1Ab, sap sucking aphids feeding on Bt corn and their honeydew were analysed for presence of Cry1Ab using ELISA. Phloem sap of Bt and non-Bt corn was collected using a newly developed technique with a microcapillary being directly inserted into the phloem tubes. Using this technique, 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 *Rhopalosiphum padi* L. and their honeydew, no Cry1Ab could be detected. In contrast, Cry1Ab was clearly detected in both larvae of the leaf chewing herbivore *Spodoptera littoralis* (Boisduval) and their faeces, showing that Cry1Ab is detectable after ingestion and excretion by herbivores. These results suggest that *R. padi* ingests or contains no or only very low concentrations of Cry1Ab in the range of the detection limit. In consequence it is hypothesized 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.

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.

Field studies were conducted in 1992 and 1993 in Hermiston, Oregon, to evaluate the efficacy of transgenic Bt potato (Newleaf(R), which expresses the insecticidal protein Cry3Aa) 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 Newleaf potato 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, bi-weekly applications of permethrin, or early- and mid-season applications of systemic insecticides (phorate and disulfoton). When compared with conventional potato plants not treated with any insecticides, the effective control of CPB by Newleaf potato plants or weekly sprays of a Bt-based formulation did not significantly impact the abundance of beneficial predators or secondary potato pests. In contrast to Newleaf potato plants or microbial Bt formulations, however, bi-weekly 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. Thus, transgenic Bt potato, Bt- based microbial formulations and systemic insecticides appeared to be compatible with the development of integrated pest management (IPM) against other potato pests such as GPA because these CPB control measures have little impact on major natural enemies. In contrast, the broad-spectrum pyrethroid insecticide (permethrin) is less compatible with IPM programs against GPA and the potato leafroll viral disease.

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.

Studies of the effects of insect-resistant transgenic plants on beneficial insects have, to date, concentrated mainly on either small-scale 'worst case scenario' laboratory experiments or on field trials. We present a laboratory method using large population cages that represent an intermediate experimental scale, allowing the study of ecological and behavioural interactions between transgenic plants, pests and their natural enemies under more controlled conditions than is possible in the field. Previous studies have also concentrated on natural enemies of lepidopteran and coleopteran target pests. However, natural

enemies of other pests, which are not controlled by the transgenic plants, are also potentially exposed to the transgene product when feeding on hosts. The reduction in the use of insecticides on transgenic crops could lead to increasing problems with such nontarget pests, normally controlled by sprays, especially if there are any negative effects of the transgenic plant on their natural enemies. This study tested two lines of insect-resistant transgenic oilseed rape (*Brassica napus*) for side-effects on the hymenopteran parasitoid *Diaeretiella rapae* and its aphid host, *Myzus persicae*. One transgenic line expressed the F-endotoxin Cry1Ac from *Bacillus thuringiensis* (Bt) and a second expressed the proteinase inhibitor oryzacystatin I (OC-I) from rice. These transgenic plant lines 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.

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

The oviposition behavior of adult insects can have a major impact on the level of exposure of their offspring to natural and artificial toxins. Pollen from Pt-corn hybrids represents a novel toxin and very little is known regarding its detection and possible avoidance by ovipositing females. The effect of corn plant proximity and corn pollen presence on the oviposition behavior of the monarch butterfly, *Danaus plexippus* (L.), was assessed in cage and flight chamber studies. The proportions of monarch eggs oviposited on milkweed plants dusted pollen from with a Bt-corn hybrid, an 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 untransformed 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. There were no significant differences in the effects of Bt corn plants or corn pollen compared with untransformed plants or pollen. These results may have important implications for the level of exposure of monarch larvae to Pt-corn pollen.

Van Dam, N. M. and Baldwin, I. T. 2001. Competition mediates costs of jasmonate-induced defences, nitrogen acquisition and transgenerational plasticity in *Nicotiana attenuata*. *Funct Ecol* **15**: 406-415.

1. Plants adjust their phenotype in response to environmental signals, but little is known about the interaction of plastic responses to simultaneously occurring environmental stresses. 2. To examine the costs of induced resistance on reproductive performance in plants subjected to other important environmental variables, resistance was elicited with a jasmonate treatment (MeJA) to one, both or neither of two *Nicotiana attenuata* plants growing competitively in either high- or low-N soils. Half the plants were subjected to leaf removal (LR). (KNO<sub>3</sub>)-N-15 was used to quantify differences in N acquisition and allocation. Transgenerational effects were measured with seed germination and seedling performance tests. 3. An induced plant competing with an uninduced plant produced significantly fewer seeds, acquired less N-15 and allocated less N-15 to seed production. Uninduced plants competing with induced plants realized a comparable fitness benefit. 4. The costs of induction were greater under high N. Plants grown under low N minimized costs by allocating significantly more N to seeds. LR decreased seed production independently of any other effect. Low N and LR both reduced germination rates. 5. The effects of MeJA on seed germination depended on competition and N supply. The differences in germination rates resulted in dramatic fitness differences among offspring. 6. *N. attenuata* plants appear to use N availability and their induced status to alter their current phenotype and their offspring's phenotype to adjust to environmental changes that occur predictably over time in their natural environment.

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 U S A* **98**: 11908-11912.

The widespread planting of corn genetically modified to produce *Bacillus thuringiensis* endotoxin has led to speculation that pollen from these fields might adversely affect nearby nontarget lepidopterans. A previous study of Bt corn engineered with Monsanto event 810 failed to detect an effect of pollen exposure on the black swallowtail, *Papilio polyxenes*, in either the field or the laboratory. Here, we report results of a field study investigating the impact of exposure to pollen from a Bt corn hybrid containing Novartis event 176 on two species of *Lepidoptera*, black swallowtails and monarch butterflies, *Danaus plexippus*. Nearly half of



the 600 monarch larvae died within the first 24 h; this and subsequent mortality was not associated with proximity to St 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, we observed a significant reduction in growth rates of black swallowtail larvae that was likely caused by pollen exposure. These results suggest that Bt corn incorporating event 176 can have adverse sublethal effects on black swallowtails in the field and underscore the importance of event selection in reducing environmental impacts of transgenic plants.

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.

The effects of transgenic herbicide-resistant soybean varieties and their corresponding weed management strategies on canopy insects were examined in studies at two locations in Iowa 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. These findings indicate that although the transgenic soybean varieties did not strongly affect insect populations, weed management systems can affect insect populations in soybean. However, this impact is likely related more to weed suppression effectiveness than to a direct effect of the herbicides on the insects.

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.

We present the first evidence that transgenic *Bacillus thuringiensis* (Bt) corn pollen naturally deposited on *Asclepias syriaca*; common milkweed, in a corn field causes significant mortality of *Danaus plexippus* L. (Lepidoptera: Danaidae) larvae. Larvae feeding for 48 h on *A. syriaca* plants naturally dusted with pollen from Bt corn plants suffered significantly higher rates of mortality at 48 h (20+/-3%) compared to larvae feeding on leaves with no pollen (3+/-3%), or feeding on leaves with non-Bt pollen (0%). Mortality at 120 h of *D. plexippus* larvae exposed to 135 pollen grains/cm<sup>2</sup> of transgenic pollen for 48 h ranged from 37 to 70%. We found no sub-lethal effects on *D. plexippus* adults reared from larvae that survived a 48-h exposure to three concentrations of Bt pollen. Based on our quantification of the wind dispersal of this pollen beyond the edges of agricultural fields, we predict 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 will likely occur on *A. syriaca* plants in corn fields or within 3 m of the edge of a transgenic corn field. We conclude that the ecological effects of transgenic insecticidal crops need to be evaluated more fully before they are planted over extensive areas.

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.

Here we show 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 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.

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

We estimated the effect of deploying Cry3A-transgenic potatoes resistant to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), 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 (*P* less than or equal to 0.05) more abundant in transgenic

treatment fields than in nontransgenic 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 treatment fields in 1995. We conclude that the deployment of pure stands of Cry3A-transgenic potatoes, 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.

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.

According to recent analyses of complete genome sequences horizontal gene transfers have played a fundamental role in bacterial evolution. Nowadays, this is efficiency of bacteria in picking up genes of surrounding organisms that rises concerns about a potential dissemination of genes from transgenic plants to soil bacteria. The only mechanism which could be involved for such gene exchanges 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. The data I present in this paper indicate that transformation-mediated gene transfers could occur in the environment and in the case of transgenic plants are mote likely to involve the prokaryotic sequences of the transgene than the remaining of the genome. 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.

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.

Insecticidal proteins produced by various subspecies of *Bacillus 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. The persistence of bound DNA has relevance to horizontal gene transfer in soil. Because of the large differences in the chemical composition and structure between these proteins and DNA, as well as between clays and humic acids, these studies can serve as models for the potential fate and effects of other biomolecules that will be introduced to soil from "factories" of transgenic plants and animals genetically engineered to produce vaccines, hormones, antibodies, toxins, pharmaceuticals, and other bioactive compounds.

Tomiuk, J., Hauser, T. P. and Bagger-Jorgensen, R. 2000. A- or C-chromosomes, does it matter for the transfer of transgenes from *Brassica napus*. *Theor Appl Genet* **100**: 750-754.

Introgression of genes from allotetraploid *Brassica napus* into its diploid wild relative *B. mpa* is generally considered to be inevitable. As a means to minimize a potential ecological risk in environments where *B. ml,a* is growing, the insertion of transgenes into chromosome regions of *B. napus* with a very low probability of transfer to backcross generations with *B. rapa* has been proposed. Recently, the progeny of four backcross generations between transgenic herbicide-tolerant *B. napus* and *B. rapa* was studied in selection experiments (Metz et al. 1997). The rapid decrease in the frequency of herbicide- tolerant plants was explained by selection against the C- chromosomes of *B. napus* in favor of the homeologous ii-chromosomes. Obviously, such C-chromosomes could be potential candidates as safe integration sites for transgenes. We considered these safety aspects using a simple population genetic model. Theory and experiments, however, do not favor the chromosomes of *B. napus* as safe candidates with respect to the introgression of transgenes into wild populations of *B. rapa*.

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 U S A* **97**: 7700-7703.

A single laboratory study on monarch butterflies has prompted widespread concern that corn pollen, engineered to express *Bacillus thuringiensis* (Bt) endotoxin, might travel beyond corn fields and cause mortality in nontarget lepidopterans. Among the lepidopterans at high potential risk from this technology is the black swallowtail butterfly. *Papilio polyxenes*, whose host plants in the midwestern U.S. are located principally in narrow strips between roads and crop fields. 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 St corn (Pioneer 34R07 containing Monsanto event 810) at the beginning of anthesis. We confirmed by ELISA that pollen from these plants contained Cry1Ab endotoxin (2.125 +/- 0.289 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<sup>2</sup>), a level that far exceeded the highest pollen density observed in the field (200 grains/cm<sup>2</sup>). We conclude that Bt pollen of the variety tested is unlikely to affect wild populations of black swallowtails. Thus, our results suggest that at least some potential nontarget effects of the use of transgenic plants may be manageable.

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.

Laboratory feeding experiments using transgenic *Bacillus thuringiensis* variety *kurstaki* (Berliner) corn plants were carried out to study the effects of *B. thuringiensis*-fed herbivorous prey on the predator *Orius majusculus* (Reuter). Host plants were a transgenic *B. thuringiensis*-expressing (Cry1Ab) corn hybrid and the corresponding untransformed isogenic *B. thuringiensis*-free corn hybrid. The herbivorous prey species used in the experiment was *Anaphothrips obscurus* (Muller), a thysanopteran pest of corn, not sensitive to Cry1Ab toxin. The objectives were to quantify the effects of *B. thuringiensis*-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 *B. thuringiensis*-fed or *B. thuringiensis*-free prey. Similarly, no significant differences in total developmental time of *O. majusculus* was detected when reared on the two different prey types. Overall mortality was low, confirming that the methodology used was appropriate. We propose this approach as an efficient standardized preregistrational testing for side effects of transgenic plants on small predators such as *Orius* spp.

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. Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. *Mol Breed* **5**: 75-83.

Transgenic crops genetically engineered for enhanced insect resistance should be compatible with other components of IPM for the pest resistance to be durable and effective. An experimental potato line was genetically engineered to express an anti-aphid plant protein (snowdrop lectin, GNA), and assessed for possible interactions of the insect resistance gene with a beneficial pest predator. These extended laboratory studies are the first 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) colonising 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 transgenic plants 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 demonstrate that expression of a lectin gene for insect resistance in a transgenic potato line can cause adverse effects to a predatory ladybird via aphids in its food chain. The significance of these potential ecological risks under field conditions need to be further evaluated.

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.

1. A field study using transgenic plants with associated recombinant micro-organisms was conducted to assess the potential effects of genetically engineered organisms on soil ecosystems. Three genotypes 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*. 2. 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. 3. 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*. 4. 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. 5. These results suggest that the genetically engineered organisms caused detectable changes in some components of the soil ecosystem. The primary effects we observed were associated with the transgenic lignin peroxidase alfalfa and included alterations in plant growth and chemistry and changes in soil chemistry and microbiology.

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 U S A* **96**: 1840-1845.

Evolving levels of resistance in insects to the bioinsecticide *Bacillus thuringiensis* (Bt) can be dramatically reduced through the genetic engineering of chloroplasts in plants. When transgenic tobacco leaves expressing Cry2Aa2 protoxin in chloroplasts were fed to susceptible, Cry1A-resistant (20,000- to 40,000-fold) and Cry2Aa2-resistant (330- to 393-fold) tobacco budworm *Heliothis virescens*, cotton bollworm *Helicoverpa* tea, and the beet armyworm *Spodoptera exigua*, 100% mortality was observed against all insect species and strains. Cry2Aa2 was chosen for this study because of its toxicity to many economically important insect pests, relatively low levels of cross-resistance against Cry1A-resistant insects, and its expression as a protoxin instead of a toxin because of its relatively small size (65 kDa). Southern blot analysis confirmed stable integration of cry2Aa2 into all of the chloroplast genomes (5,000-10,000 copies per cell) of transgenic plants. Transformed tobacco leaves expressed Cry2Aa2 protoxin at levels between 2% and 3% of total soluble protein, 20- to 30-fold higher levels than current commercial nuclear transgenic plants. These results suggest that plants expressing high levels of a nonhomologous Bt protein should be able to overcome or at the very least, significantly delay, broad spectrum Bt-resistance development in the field.

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.

A field release of genetically engineered potato plants 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 analyzed for two T4-lysozyme-expressing lines, a transgenic control and a non-transgenic line, 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 bacteria (percentage of auxin [indole-3-acetic acid = IAA]-producing isolates) 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. This is the first study including plant-associated bacteria responsible for plant growth and health and provides an example for performing risk assessment studies for transgenic plants under a variety of environmental conditions. (C) 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Purrlington, C. B. and Bergelson, J. 1999. Exploring the physiological basis of costs of herbicide resistance in *Arabidopsis thaliana*. *Am Nat* **154**: S82-S91.

For some resistance traits identified in crop and weed species, plant physiologists have detailed

knowledge of the mechanism of gene action that distinguishes the physiology of resistant and susceptible genotypes. Such information could be useful to those evolutionary biologists interested in coupling the genetics and physiology of resistance mutations with data on the relative fitness of resistant and susceptible genotypes. In previous work, we have shown that the lifetime seed production of chlorsulfuron-resistant *Arabidopsis thaliana* was substantially lower than that of susceptible plants, and here we explore potential physiological reasons for the fitness reduction. In addition, we highlight several methodological issues that are essential when using transgenic technology to explore fitness trade-offs.

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.

Transgenic wheat plants containing the gene encoding snowdrop lectin (*Galanthus nivalis* agglutinin; GNA) under the control of constitutive and phloem-specific promoters were generated through the particle bombardment method. Thirty-two independently derived plants were subjected to molecular and biochemical analyses. Transgene integration varied from one to twelve estimated copies per haploid genome, and levels of GNA expression from 0 to ca. 0.2% of total soluble protein were observed in different transgenic plants. Seven transgenic plants were selected for further study. Progeny plants from these parental transformants were selected for transgene expression, and tested for enhanced resistance to the grain aphid (*Sitobion avenae*) by exposing the plants to nymphal insects under glasshouse conditions. Bioassay results show 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. We propose that transgenic approaches using insecticidal genes such as gna in combination with integrated pest management present promising opportunities for the control of damaging wheat pests.

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.

The equilibrium adsorption and binding of the active toxin from *Bacillus 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. The content of carboxyl groups and the degree of polymerization (E4/E6) did not appear to influence significantly the differential binding. 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 (LC50) 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. The result of these studies indicate that the toxins from *B. thuringiensis* introduced in transgenic plants and microbes could persist, accumulate, and remain insecticidal in soil as a result of binding to humic acids, as well as on clays, as previously described. This persistence could pose a hazard to non-target organisms and enhance the selection of toxin- resistant target species. (C) 1998 Elsevier Science Ltd. All rights reserved.

Harada, S., Nakata, K., Tanaka, H. and Ishiguro, Y. 1998. Environmental risk evaluation of genetically modified tomatoes introduced the antisense polygalacturonase gene. *Breed Sci* **48**: 207-212.

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.

Laboratory feeding experiments using transgenic *Bacillus thuringiensis* variety *kurstaki* (Berliner) corn plants have been carried out to study the effects of *B. thuringiensis*-fed herbivores (i.e., prey), on the predator *Chrysoperla carnea* Stephens. Host plants were a transgenic *B. thuringiensis*- expressing (CryIAb) corn hybrid and the corresponding untransformed, *B. Thuringiensis*-free corn hybrid. Two different prey species were used in the experiments, 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 *B. thuringiensis*-fed prey on chrysopid immature development and to determine whether observed effects were caused by sick, suboptimal prey (indirect effects) or associated with *B. thuringiensis*-related causes (direct effects). Mean total immature mortality for chrysopid larvae raised on *B. thuringiensis*-fed prey was 62% compared with 37% when raised on *B. thuringiensis*-free prey. There was no significant difference in mortality between chrysopid larvae reared on *B. thuringiensis*-fed *O. nubilalis* or *B. thuringiensis*-fed *S. littoralis*. Similarly, no significant difference in mortality was detected when chrysopid larvae were raised on *B. thuringiensis*-free *O. nubilalis* or *B. thuringiensis*-free *S. littoralis*. Development time of chrysopid larvae was prolonged when *B. thuringiensis*-fed *O. nubilalis* was given to the predators but not for *B. thuringiensis*-fed *S. littoralis*. Although some unnoticed adverse effects in *S. littoralis* may have occurred because of the *B. thuringiensis* corn, our results suggest that the reduced fitness of chrysopid larvae was associated with *B. thuringiensis*. The prolonged development time of chrysopid larvae raised on *B. thuringiensis*-fed *O. nubilalis* was probably because of a combined effect of *B. thuringiensis* exposure and nutritional deficiency caused by sick prey.

Moenne-Loccoz, Y., Powell, J., Higgins, P., Britton, J. and O'Gara, F. 1998. Effect of the biocontrol agent *Pseudomonas fluorescens* F113 released as sugarbeet inoculant on the nutrient contents of soil and foliage of a red clover rotation crop. *Biol Fertil Soils* **27**: 380-385.

The biocontrol agent *Pseudomonas fluorescens* F113, which produces the antimicrobial compound 2,4-diacetylphloroglucinol and inhibits soil-borne phytopathogenic fungi, might also affect non-target resident microorganisms involved in the cycling of soil nutrients. Analyses of soil nutrients and crop foliage were used to assess possible residual effects of the pseudomonad on soil fertility. The Bandon field site was sown in 1994 with sugarbeet seeds that were either untreated, inoculated with the spontaneous rifampicin-resistant mutant F113Rif, or treated with chemical fungicides. The field was cropped with uninoculated red clover in 1995. The inoculant was recovered at only 2.0 log CFU/root system at the first harvest of clovers, and the F113Rif treatment influenced 5 of 17 levels of readily available nutrients determined by electro-ultrafiltration (EUF) analysis of soil. However, this effect was essentially negligible since it was (1) small in magnitude and of little agronomic significance when compared with reference EUF levels, (2) similar in part to those of the commercial seed treatment and other farming practices followed at Bandon, and (3) not statistically significant when data were analysed at the plot level rather than using results from individual soil samples within plots. Clover leaf composition was investigated at two harvests. The F113Rif treatment had no effect on the 20 foliage parameters studied except a small (15%) transient decrease in foliage chlorine at the first harvest. EUF soil analysis and foliage analysis will be useful in future risk assessment studies of genetically modified derivatives of F113.

Donegan, K. K., Seidler, R. J., Fieland, V. J., Schaller, D. L., Palm, C. J., Ganio, L. M., Cardwell, D. M. and Steinberger, Y. 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 microarthropod populations. *J Appl Ecol* **34**: 767-777.

1. To evaluate the potential effects of genetically engineered (transgenic) plants on soil ecosystems, litterbags containing leaves of non-engineered (parental) and transgenic tobacco plants were buried in field plots. The transgenic tobacco plants were genetically engineered to constitutively produce proteinase inhibitor I, a protein with insecticidal activity. 2. 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. 3. The proteinase inhibitor concentration in the transgenic plant 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 transgenic plant litter was comparable to that of the parental plant litter on sample day 0, it became significantly lower over the course of the experiment. 4. Nematode populations in the soil surrounding the transgenic plant litterbags were greater than those in the soil surrounding parental plant litterbags and had a different trophic group composition, including a significantly higher ratio of fungal feeding to bacterial feeding nematodes on sample day 57. In contrast, Collembola populations in the soil surrounding the transgenic plant litterbags were significantly lower than in the soil surrounding parental plant litterbags. 5. Our results demonstrated that under field conditions proteinase inhibitor remained immunologically active in buried transgenic plant litter for at least 57 days and that decomposing parental and transgenic plant litter differed in quality (carbon content) and in the response of exposed soil organisms (Collembola and nematodes).

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.

To obtain durable and broad-spectrum resistance against plant pathogens, plants are transformed with genes coding for antimicrobial proteins from plant, animal or microbial origin. An obvious concern is that increased levels of these antimicrobial compounds affect not only the target pathogen, but also beneficial micro-organisms such as mycorrhizae, rhizobia and other micro-organisms involved in plant health, litter decomposition and nutrient cycling. This literature study focuses on effects of these transgenic plants on the non-target saprophytic soil microflora. Transgenic plants that constitutively express proteins with potential antifungal and/or antibacterial activity, can reduce activities of specific soil-borne plant pathogens in the rhizosphere. Reports on non-target effects on the saprophytic soil microflora are scarce and incomplete, and mainly focused on mycorrhizal symbiosis. Constitutive expression of antifungal pathogenesis-related proteins in tobacco in most cases did not affect 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 non-target effects can occur. Concerning other members of the plant-beneficial rhizosphere microflora, it can be assumed that they will come into contact with the transgenic product. By natural wounding, senescence and sloughing-off of root cells, at least some of the antimicrobial protein(s) will be released in the rhizosphere. Despite proteolytic activity of the rhizosphere microflora, part of the protein can remain active due to protective adsorption to clay minerals or humic components.

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.

One of the concerns associated with the field testing and agronomic-scale release of transgenic crops is the potential for the pollen-mediated escape of engineered genes into naturally occurring populations of wild relatives. While border rows have been used frequently to restrict the pollen-mediated escape of engineered genes from held trials, the efficacy of this approach has been little studied. To test the effectiveness of border plantings, isogenic lines of cucumber (*Cucumis sativus* L.) differing for the seedling marker trait blunt leaf apex (bla) were planted in four designs: 1 m(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 outcrossing to a single satellite, indicating that the effectiveness of borders was influenced by both the relative numbers of donor plants and by environmental variables.

Metz, P. L. J. and Nap, J. P. 1997. A transgene-centred approach to the biosafety of transgenic plants: Overview of selection and reporter genes. *Acta Bot Neerl* **46**: 25-50.

Omer, A. D., Granett, J., Dandekar, A. M., Driver, J. A., Uratsu, S. L. and Tang, F. A. 1997. Effects of transgenic petunia expressing *Bacillus thuringiensis* toxin on selected lepidopteran pests. *Biocontrol Sci Technol* **7**: 437-448.

Toxicity of 11 transgenic petunia lines expressing the CryIA (c) insecticidal crystal protein of *Bacillus thuringiensis* subsp. *kurstaki* was investigated using lepidopteran neonates *Spodoptera exigua*, *Trichoplusia ni* and *Manduca sexta*. Mortality of *S. exigua*, *T. ni* and *M. sexta* varied within and among transgenic petunia lines. Bioassay results demonstrated that levels of CryIA (c) expression obtained in 7 out of the 11 transgenic petunia lines produced at least 50% mortality in *S. exigua* and *T. ni*, and all 11 transgenic lines produced more than 80% mortality in *M. sexta*. Demographic analysis of the biological impact of transgenic petunia on *S. exigua* revealed that sub-lethal feeding on transgenic petunia significantly reduced larval weight and prolonged larval and pupal development times. Continuous feeding on transgenic petunia significantly reduced lifetime fecundity, egg hatch and longevity in female and male moths. Compared with insects fed continuously on non-transgenic petunia, lifetime fecundity and net reproductive rate were reduced by 58 and 84% in insects fed continuously on transgenic petunia respectively. Mean generation time was 8 days longer for insects fed continuously on transgenic petunia than for insects fed on non-transgenic petunia. Ovipositional attractiveness of transgenic petunia to *S. exigua* with respect to non-transgenic tomato or lettuce plants was similar, suggesting that petunia/tomato and petunia/lettuce may not be effective trap-cropping combinations. The potential and implications of using transgenic petunia as trap plants interplanted with crop plants for management of lepidopteran pests in the field are discussed.

Picard-Nizou, A. L., Grison, R., Olsen, L., Pioche, C., Arnold, G. and Pham-Delegue, M. H. 1997. Impact of proteins used in plant genetic engineering: Toxicity and behavioral study in the honeybee. *J Econ Entomol* **90**: 1710-1716.

Genetically modified oilseed rape, *Brassica napus* L., expressing pest and fungi resistance has been assessed for its impact: on the environment before field testing and placing on the market. The evaluation of rape lines for their innocuousness to beneficial insects such as the pollen bee, *Apis mellifera* L., were investigated because changes in the composition of nectar and pollen may occur that affect plant-honeybee interactions. The effects of these giants on bees were evaluated by acute toxicity testing of the gene products and behavioral studies of bees. Acute toxicity was determined by evaluating the mortality over 24- and 48-h periods after ingestion or injection of the gene products. For the bee behavioral study, methods used to study learning processes and foraging behavior were adapted to test the effect of the gene products at both individual and colony levels under confined conditions. The study focused on the effects of proteins inducing resistance to pest insects, cowpea trypsin inhibitor (CpTI), and resistance to fungi, chitinase, and beta-1,3 glucanase. Under our experimental conditions, the 3 proteins were shown to be nontoxic at the doses tested. At the individual level, behavior experiments, based on a conditioned proboscis extension response had the following 3 effects depending on the protein tested: (1) chitinase did not affect learning performance; (2) beta-1,3 glucanase affected the level of conditioned responses, with the extinction process occurring more rapidly as the concentration increased and (3) CpTI induced marked effects in both conditioning and testing phases, especially in high concentrations. The decrease in learning performance induced by CpTI observed at the individual level was confirmed at the colony level. Given their relative simplicity, it is suggested that such bioassays could be used for screening in the development of genetically modified plants.

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.

Laboratory studies determined the effects of feeding corn, *Zea mays* L., pollen expressing a CryIAb protein derived from *Bacillus thuringiensis* (Berliner) subsp. *kurstaki* on 3 predatory species: *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), *Orius insidiosus* Say (Heteroptera: Anthrenidae), and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae). No acute detrimental effects of the transgenic *B. thuringiensis* pollen (CryIAb protein) on preimaginal development and survival were observed among these predators. The following percentage survival values ( $\pm$  SE) were observed: *C. maculata*, 89  $\pm$  2.2% (*B. thuringiensis* corn pollen), 69  $\pm$  5.9% (non-*B. thuringiensis* corn pollen); *O. insidiosus*, 63  $\pm$  12% (*B. thuringiensis* corn pollen), 44  $\pm$  10.2% (non-*B. thuringiensis* corn pollen); and *C. carnea*, 49  $\pm$  3.5% (*B. thuringiensis* and non-*B. thuringiensis* corn pollen). No detrimental effects were observed in the abundance of *ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) predators (coccinellids, anthrenids, chrysopids) in *B. thuringiensis* corn compared with non-*B. thuringiensis* corn during 2 yr of field evaluations. Predator numbers observed before, during, and after pollen shed suggest that *B. thuringiensis* corn pollen will not affect natural enemy movement in corn. Additional studies are needed to test for chronic and reproductive effects over several generations before concluding that transgenic *B. thuringiensis* corn pollen has no effect on insect predators.

Sims, S. R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein. Effects on Lepidoptera, Diptera, and non-target arthropods. *Southw Entomol* **22**: 395-404.

Purified CryIIA insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki* was tested against one isopod (Crustacea, Isopoda) and 35 insect species representing the orders Coleoptera, Collembola, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, and Orthoptera. All tests were conducted using diet-incorporation feeding bioassays. Only Lepidoptera and Diptera (Culicidae) were sensitive to the CryIIA protein. The most susceptible Lepidoptera species, *Anticarsia gemmatilis* was >12500-fold more sensitive ( $LC_{50}$  = 0.016  $\mu$ g/ml) than *Agrotis ipsilon* and *Spodoptera frugiperla* ( $LC_{50}$  values >200  $\mu$ g/ml). Sublethal concentrations of CryIIA protein reduced the larval growth of all Lepidoptera species. Among the Culicidae, the most susceptible species, *Anopheles quadrimaculatus* ( $LC_{50}$  = 0.037  $\mu$ g/ml) was >500-fold more sensitive than *Culex pipiens* ( $LC_{50}$  >200  $\mu$ g/ml). Other Diptera (*Musca* and *Drosophila*) and non-lepidoptera species were not affected by ingestion of the CryIIA protein. These data confirm that CryIIA protein has biological activity specific for Lepidoptera and Diptera, and demonstrate that considerable variability in species susceptibility exists within these orders.

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.



Rapeseed *Brassica napus* L. transgenic for a *Bacillus thuringiensis* (Bt) transgene was developed and was shown to be 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 transgenic and nontransgenic 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 plants, both transgenic, 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 nontransgenic plants relative to Bt-transgenic 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. This is the first report of a field study demonstrating the effect of a fitness-increasing transgene in plants.

Wilkinson, J. E., Twell, D. and Lindsey, K. 1997. Activities of CaMV 35S and nos promoters in pollen: Implications for field release of transgenic plants. *J Exp Bot* **48**: 265-275.

The expression of foreign genes in pollen may pose potential problems in the field release of transgenic plants, since pollen represents a route whereby foreign genes and their products may escape into the wider environment. The possible risks posed by crosshybridization with wild relatives have been extensively explored, but problems that may arise due to the expression of foreign gene products in pollen have not been so widely studied. The activities of the CaMV 35S and nos promoters in pollen in populations of stably transformed plants and in transient expression analysis are described. These promoters are commonly used in all areas of plant molecular biology research and their expression patterns will be of interest to those involved in field release studies. The results show that both promoters had no detectable pollen activity in *Arabidopsis*, but both showed activity in tobacco pollen. The CaMV 35S-gus gene fusion showed heritable expression levels in tobacco pollen of up to a maximum of 64.6 pmol 4-MU min<sup>-1</sup> mg<sup>-1</sup> total protein, nos promoter activity in transgenic tobacco pollen was highly variable, with GUS activities ranging from undetectable levels up to 2561 pmol 4-MU min<sup>-1</sup> mg<sup>-1</sup> total protein within the transgenic population. Histochemical staining of anther sections from 10- 12 mm buds revealed that the CaMV 35S promoter had some activity in the vascular bundle, stomium and tapetum, while GUS expression from the nos promoter in sporophytic tissues was confined entirely to the stomium.

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.

Methods are reported for testing the effects of toxins or small molecules [*Bacillus thuringiensis* ssp. kurstaki delta- endotoxins HD-1 CryIA(b) and HD-73 CryIA(c)] in plants (cotton and potato) on 2 nontarget soil arthropods, a collembolan, *Folsomia candida* Willem, and an oribatid mite, *Oppia nitens* Koch. Time to oviposition, egg production, and Final body length were unaffected when *F. candida* were fed residues of transgenic cotton lines #81 or #249. Total production of *O. nitens* adults and nymphs was unaffected by feeding on leaves of both transgenic cotton lines. with *B. thuringiensis* ssp. tenebrionis CryIIIA in potato, no differences were seen in the 3 indices for *F. candida*. Overall, transgenic toxin sources had no negative effects, but similar tests with cadmium as a positive control showed a concentration-rate response of decreased reproduction.

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 Lond Ser B-Biol Sci* **263**: 1659-1663.

Evolutionary biologists have long attributed polymorphisms in resistance status to fitness costs of resistance traits. Nevertheless, pleiotropic fitness costs of resistance have been notoriously difficult to detect. We have transformed *Arabidopsis thaliana* with a mutant acetolactate synthase gene that confers resistance to the herbicide, chlorsulfuron. Our experiment revealed a 34% reduction in the lifetime seed production of transgenic, herbicide resistant *Arabidopsis thaliana* relative to their susceptible null-segregants. Our experimental design allows us to conclude that this fitness cost of resistance is caused by the pleiotropic effect of the introduced acetolactate synthase gene rather than other potential costs associated with the plasmid or mutational changes induced by plant transformation. In addition, we can attribute the cost of resistance to the presence of the resistance gene rather than an increase in gene dosage. The implications of these results for the risk assessment of transgenic crops are discussed.

Cruywagen, G. C., Kareiva, P., Lewis, M. A. and Murray, J. D. 1996. Competition in a spatially heterogeneous environment: Modelling the risk of spread of a genetically engineered population. *Theor Popul Biol* **49**: 1-38.

In recent years regulations have been developed to address the risks of releasing genetically engineered organisms into the natural environment. These risks are generally considered to be proportional to the exposure multiplied by the hazard. Exposure is, in part, determined by the spatial spread of the organisms, a component of risk suited to mathematical analysis. In this paper we examine a mathematical model describing the spread of organisms introduced into a heterogeneous environment, focusing on the risk of spread and plausibility of containment strategies. Two competing populations are assumed, one the natural species and the other an engineered species or strain, both of which move randomly in a spatially heterogeneous environment consisting of alternating Favourable and unfavourable patches. The classical Lotka-Volterra competition model with diffusion is used. Analyses of the possible spread and invasion of engineered organisms are thus reduced to finding periodic travelling wave solutions to the model equations. We focus on whether a very small number of engineered organisms can spatially invade a natural population. Initially we investigate the problem for spatially periodic diffusion coefficients and demonstrate that, under the right circumstances and a large enough unfavourable patch, invasion does not succeed. However, if spatially periodic carrying capacities are assumed along with spatially varying diffusion rates, the situation is far more complex. In this case containment of the engineered species is no longer only a simple function of the unfavourable patch length. BS using perturbation solutions to the nonuniform steady states. approximate invasion conditions are obtained. (C) 1996 Academic Press, Inc.

Donegan, K. K., Schaller, D. L., Stone, J. K., Ganio, L. M., Reed, G., Hamm, P. B. and Seidler, R. J. 1996. Microbial populations, fungal species diversity and plant pathogen levels in field plots of potato plants expressing the *Bacillus thuringiensis* var *tenebrionis* endotoxin. *Transgenic Res* **5**: 25-35.

The environmental release of genetically engineered (transgenic) plants may be accompanied by ecological effects including changes in the plant-associated microflora. A field release of transgenic potato plants that produce the insecticidal endotoxin of *Bacillus thuringiensis* var. *tenebrionis* (Bt) 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 (green, yellow and brown) were compared over the growing season (sample days 0, 21, 42, 63 and 98) for transgenic potato plants, commercial Russet Burbank potato plants treated with systemic insecticide (Di-Syston) and commercial Russet Burbank potato plants treated with microbial Bt (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. Total bacterial populations on brown leaves on sample day 21 and on green leaves on sample day 42 were significantly higher on the transgenic potato plants. Total fungal populations on green leaves on sample day 63 were significantly different among the three plant types; lowest levels were on the commercial potato plants treated with systemic insecticide and highest levels were on the commercial potato plants treated with microbial Bt. Differences in fungal species assemblages and diversity were correlated with sampling dates, but relatively consistent among treatments. *Alternaria alternata*, a common saprophyte on leaves and in soil and leaf litter, was the most commonly isolated fungus species for all the plant treatments. Rhizosphere populations of the soilborne pathogens *Pythium* spp., *Fusarium* spp. and *V. dahliae* did not differ between the transgenic potato plants and the commercial potato plants treated with systemic insecticide. The incidence of tuber infection at the end of the growing season by the plant pathogen *V. dahliae* was highest for the transgenic potato plants but this difference was related to longer viability of the transgenic potato plants. This difference in longevity between the transgenic potato plants and the commercial + systemic insecticide potato plants also made comparison of the incidence of PVY and PLRV problematic. Our results indicate that under field conditions the microflora of transgenic Bt-producing potato plants differed minimally from that of chemically and microbially treated commercial potato plants.

LaSota, L. R. 1996. Evaluation by the United States Environmental Protection Agency of pesticidal substances produced in plants. *Field Crop Res* **45**: 181-186.

The legislative mandates and proposed scope of the U.S. Environmental Protection Agency's (EPA) regulation of pesticides produced in plants are described. The first three Experimental Use Permits (EUPs) granted by EPA for field testing of plant-pesticides were for delta endotoxins from *Bacillus thuringiensis* expressed in cotton (*Gossypium hirsutum* L.), potato (*Solanum tuberosum* L.) and maize (*Zea mays* L.). In each instance, an environmental-fate assessment by the Agency found no significant risk of gene expression beyond the spatial and temporal limits of the proposed field tests.

Lavigne, C., Godelle, B., Reboud, X. and Gouyon, P. H. 1996. A method to determine the mean pollen dispersal of individual plants growing within a large pollen source. *Theor Appl Genet* **93**:

1319-1326.

Pollen dispersal has been recently focused on as a major issue in the risk assessment of transgenic crop plants. 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. We report here on a method based on the use of Fourier transforms by which the pollen dispersal of a single, average individual can be obtained from data using large plots as pollen sources, thus allowing the estimation of the probability of long-distance dispersal for single plants. This method is subsequently implemented on simulated data to test its susceptibility to random noise and edge effects. Its conditions of application and value for use in ecological studies, in particular risk assessment of the deliberate release of transgenic plants, are discussed.

Matzk, A., Mantell, S. and Schiemann, J. 1996. Localization of persisting agrobacteria in transgenic tobacco plants. *Mol Plant-Microbe Interact* **9**: 373-381.

The persistence of *Agrobacterium tumefaciens* cells of strain C58C1 in transformed tobacco plants (*Nicotiana tabacum*) was assessed over a period of 12 months post-transformation in vitro, and 3 to 6 months thereafter ex vitro. Three main approaches, i.e., enrichment culture followed by identification methods, tissue-print immunoblotting, and scanning electron microscopy, were combined to investigate the persistence of the agrobacteria used for transformation. Agrobacteria cells were present on the surface and within the tissues of in vitro shoot cultures at 12 months post-transformation. Moreover, persisting agrobacteria were detectable in these transgenic tobacco plants grown ex vitro in soil for at least 3 to 6 months. Results of both tissue-print immunoblotting and enrichment culture experiments showed that agrobacteria were unevenly distributed in weaned transgenic tobacco, with the majority of agrobacteria cells being located at the stem base and in the roots of the plants. There was no evidence of agrobacteria being present in either flowers or mature seeds. The implications of these results are discussed in the context of the potential risks of inadvertent introduction of persisting transgenic agrobacteria into the environment during the release of transgenic plants.

Raybould, A. F., Goudet, J., Mogg, R. J., Gliddon, C. J. and Gray, A. J. 1996. Genetic structure of a linear population of *Beta vulgaris* ssp *maritima* (sea beet) revealed by isozyme and RFLP analysis. *Heredity* **76**: 111-117.

Knowledge of the genetic structure of plant populations is necessary for the understanding of the dynamics of major ecological processes. It also has applications in conservation biology and risk assessment for genetically modified crops. This paper reports the genetic structure of a linear population of sea beet, *Beta vulgaris* ssp. *maritima* (the wild relative of sugar beet), on Furzey Island, Poole Harbour. The relative spatial positions of the plants were accurately mapped and the plants were scored for variation at isozyme and RFLP loci. Structure was analysed by repeated subdivision of the population to find the average size of a randomly mating group. Estimates of  $F_{ST}$  between randomly mating units were then made, and gave patterns consistent with the structure of the population being determined largely by founder effects. The implications of these results for the monitoring of transgene spread in wild sea beet populations are discussed.

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.

Genetic engineering offers the opportunity to generate plants with useful new traits conferred by genes originating from a variety of organisms. The objectives of this study were to establish methods for investigating persistence of recombinant plant marker DNA after introduction into soil and to collect data from controlled laboratory test systems. As a model system, we studied the stability of DNA encoding recombinant neomycin phosphotransferase II (rNPT-II), a neomycin/kanamycin resistance marker, used in plant genetic engineering. The recombinant nature of the target (i.e. fusion of nopaline synthase promoter and NPT-II coding region) allowed us to design a rNPT-II-specific PCR primer pair. DNA preparation and quantitative PCR protocols were established. 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. We hypothesize that this DNA may have been adsorbed to soil particles and was protected from complete degradation.

Cresswell, J. E., Bassom, A. P., Bell, S. A., Collins, S. J. and Kelly, T. B. 1995. Predicted pollen dispersal by honey-bees and three species of bumble-bees foraging on oil-seed rape: A comparison of three models. *Funct Ecol* **9**: 829-841.

1. Comparisons among animal pollinators of the spatial distributions of pollen that they produce have typically been made among morphologically disparate pairs of species. In contrast, we investigated the potential extent of pollen dispersal by honey-bees (*Apis mellifera*) and bumble-bees (*Bombus lapidarius*, *B. pascuorum* and *B. terrestris*) foraging in rows of oil-seed rape (*Brassica napus* cv Westar). 2. We estimated the pollen carryover attributable to individual bees by using particulate fluorescent dye as a pollen analogue. Most of the dye was deposited at the first few flowers probed and smaller proportions were deposited up to the 20th successively probed flower. We found no significant interspecific differences in dye carryover mediated by individuals of *A. mellifera*, *B. lapidarius* and *B. terrestris* with respect to either the amount deposited or the rate of decline in deposition across successively probed flowers. We present evidence that the dye produced a reasonably good analogue of pollen transfer. 3. Bees typically flew from one plant to another nearby in the same row and were strongly directional in their movements. Bee species differed significantly in their movement patterns, with *B. terrestris* having the greatest mean move length and directionality. 4. We used three kinds of model (a numerical simulation and two different sets of diffusion-advection equations) to attempt to emulate bee movements. The predictions from all models were reasonably consistent with the observed bee movements, although the numerical simulation invariably made the most accurate predictions, particularly over the first few moves. 5. Predicted bee movements were combined with least-squares models of dye deposition to estimate the spatial dispersal of pollen by each bee species. All models ranked the bees in the same order of decreasing effectiveness in dye dispersal: *B. terrestris*, *A. mellifera*, *B. lapidarius*, *B. pascuorum*, although, except for long-distance dispersal, there was only minor variation among the bee species in the predicted extents of dye dispersal (e.g. the models predicted that the median dispersal distance would be approximately two intervening plants irrespective of the species of bee). Overall, the consensus of the models' predictions is that most of the pollen from a source plant is deposited on immediate neighbours, but that long-distance pollen dispersal in this system extends over approximately 20- 40 intervening plants from the originating plant, depending on the identity of the pollinator.

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.

Applications for the commercial release of herbicide-resistant crops, most of them transgenic, are likely to become more frequent in the coming years. The ecological concerns raised by their large scale use call for risk-assessment studies. One of the major issues in such studies is the relative fitness of the resistant line compared to the susceptible when no herbicide is applied since this will largely determine the long-term fate of the resistance gene outside of the field. Here we report on a comparison of a sulfonylurea-resistant line of white-chicory regenerated from a non-mutagenized cell culture with a supposedly isogenic susceptible biotype. 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 suggests 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.

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.

*Bacillus thuringiensis* (Bt) crystal toxins are safe biological insecticides, but have short persistence and are poorly effective against pests that feed inside plant tissues. Production of effective levels of these proteins in plants has required resynthesis of the genes encoding them. We report that amplification of an unmodified cryIA(c) coding sequence in chloroplasts 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*. Since the plastid transgenes are not transmitted by pollen, this report has implications for containment of Bt genes in crop plants. Furthermore, accumulation of insecticidal protein at a high level will facilitate improvement in the management of Bt resistant insect populations.

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.

The impact of genetically modified oilseed rape (*Brassica napus* L.) on the foraging behaviour of honey bees (*Apis mellifera* L.) was evaluated on two different lines transformed to express 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 plants were analysed in terms of nectar quantity and quality (nectar volume and sugar content). The results showed no effects on bee foraging behaviour due to the modification of the genome of these plants by the introduction of a chitinase gene even though some differences between lines were found in the nectar. The methods applied in this original approach for the evaluation of the impact of genetically modified oilseed rape were shown to be sufficiently sensitive to detect changes in bee behaviour resulting from differences between plants.

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.

Purified preparations of a *Bacillus thuringiensis* var. *kurstaki* CryIA(c) protein equivalent to the insecticidal protein produced by transgenic cotton were tested against 14 species of insects: Coleoptera; *Anthonomus grandis* Boheman, *Diabrotica undecimpunctata howardi* Barber, *Leptinotarsa decemlineata* (Say), *Hippodamia convergens* Guerin-Meneville, Diptera; *Aedes aegypti* (L.), Homoptera; *Myzus persicae* (Sulzer), Hymenoptera; *Apis mellifera* L., *Nasonia vitripennis* (Walker), Lepidoptera; *Ostrinia nubilalis* (Hubner), *Manduca sexta* (L.), *Helicoverpa tea* (Boddie), *Heliothis virescens* (Fabr.), Neuroptera; *Chrysopa carnea* Stephens, 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 or 50 µg/ml of the trypsin-resistant CryIA(c)T 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 the four species of Lepidoptera had > 50% mortality to both the CryIA(c) and CryIA(c)T proteins. The mortality of non-lepidoptera species exposed to the proteins did not differ significantly from control mortality. Data from this and other studies support the conclusion 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.

Andow, D. A. 1994. Community Response to Transgenic Plant Release - Using Mathematical-Theory to Predict Effects of Transgenic Plants. *Mol Ecol* **3**: 65-70.

Predicting the potential effects of introductions of plants on the structure of plant communities has been elusive. I suggest that mathematical models of resource competition might be useful for identifying categories of plants that either are unlikely to alter community structure or that have the potential for altering community structure. Assuming that the transgenic plant will escape and establish viable populations in nontarget habitats, this theory suggests that species that have a high minimum resource requirement are unlikely to alter community structure. The theory is elaborated to evaluate the potential effects on community structure of transgenic plants with resistance to primary consumers. Results indicate that the greatest reduction in the minimum resource requirement caused by resistance will occur when consumers are consuming enough plant biomass that the plant can no longer grow. If resistance to such a consumer were incorporated into a plant, it could lower the minimum resource requirement sufficiently that a transgenic plant would be able to alter community structure substantially. Examples of introductions of exotic plants, plant pathogens, and insect herbivores are given to support the conceptual basis of the theory. Not all transgenic plants with resistance, however, have the potential to alter community structure. Resistance to primary consumers that strongly reduce the biomass producing ability of a plant will probably be able to alter community structure, whereas resistance that reduces most other types of yield loss is less likely to alter community structure. The theory should be elaborated to incorporate more-realistic assumptions, such as those regarding reproduction, dormancy, and dispersal of the transgenic plants, and provide more detailed characterization of the potential hazard of transgenic plants to plant communities.

Fitt, G. P., Mares, C. L. and Llewellyn, D. J. 1994. Field-Evaluation and Potential Ecological Impact of Transgenic Cottons (*Gossypium-Hirsutum*) in Australia. *Biocontrol Sci Technol* **4**: 535-548.

The first field trials in Australia of transformed cottons expressing the CryIA(b) insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki* (Bt) were completed during the 1992-93 season. The trials showed good efficacy of the plants against field populations of *Helicoverpa*, but there were indications of a

declining level of Bt expression once plants began to senesce. Laboratory assays showed that larger instars could survive on the transgenic tissues although their growth was severely retarded. The introduction of Bt transgenic cottons may have several ecological impacts, apart from their direct impact on target pests. These include the risk of resistance development, effects on beneficial and non-target arthropod species and changes in pest status associated with altered patterns of pesticide usage. Chief among the potential pests are sucking insects (e.g. Miridae) which appear not to be regulated by beneficial agents and are currently suppressed by sprays applied for *Helicoverpa*. Transgenic Bt plants suffered heavily from mite attack in unsprayed plots. The potential importance of these ecological changes is discussed. Resistance management for transgenic cotton in Australia is likely to depend on a refuge strategy complemented by high expression of Bt protein in the plants, building on the successful implementation of the Insecticide Resistance Management Strategy to ensure compliance with appropriate refuge options. The development and assessment of these resistance management strategies is a key component of pre-commercialization studies in Australia.

Jepson, P. C., Croft, B. C. and Pratt, G. E. 1994. Test Systems to Determine the Ecological Risks Posed by Toxin Release from *Bacillus-Thuringiensis* Genes in Crop Plants. *Mol Ecol* **3**: 81-89.

Procedures for the selection of species for ecotoxicological risk assessment of *Bacillus thuringiensis* (Bt) gene products in the epigeal and hypogeal environments are proposed. Although species can be selected on the basis of ecological realism and functional importance, the number of organisms requiring testing and the nature of the test procedures remain uncertain with such a selectively toxic material. The heterogeneity of the soil environment, the stratification of plant material at different stages of breakdown and decomposition and the aggregation and patterns of movement of the soil fauna and flora impose problems for the design of ecologically relevant test methods. Similarly, the impact upon beneficial invertebrates, if toxic effects are detected, will be mediated by the scale and pattern of transgenic plant release in the fragmented agricultural landscape. To properly assess the ecological risks posed by a widely released toxin with a narrow spectrum of effects, a combination of laboratory tests, field experiments and longer-term monitoring will be required.

Tabei, Y., Oosawa, K., Nishimura, S., Watanabe, S., Tsuchiya, K., Yoshioka, K., Fujisawa, I. and Nakajima, K. 1994. Environmental Risk-Evaluation of the Transgenic Melon with Coat Protein Gene of Cucumber Mosaic-Virus in a Closed and Semiclosed Greenhouse. *Breed Sci* **44**: 207-211.

Environmental risk evaluation of transgenic melon plants introduced with the coat protein gene of cucumber mosaic virus was carried out in a closed and a semi-closed greenhouse as described in previous studies (Tabei et al. 1994). 1. The possibility of harmful influences on environment due to compounds produced by transgenic melon were examined. The following compounds were compared between the transgenic melon and non-transgenic melon plants: (1) phenolic acids, generally considered as allelochemical substances, produced in the plant body and secreted from the root, and (2) production of volatile compounds released from the plant into the atmosphere. Germination ratio, root length and fresh weight of cabbage in the soil used for the cultivation of either transgenic or non-transgenic melon plants, and in the soil mixed with dry powder prepared from these respective melon plants. Specific phenolic acids and volatile compounds were not detected from transgenic melon plants. There were no differences for germination ratio, root length and fresh weight of cabbage between transgenic and non-transgenic melon. These results suggested that transgenic melon plants did not produce any specific products influencing environment and other plants. 2. The influence of transgenic melon cultivation on the soil microflora was investigated in a semi-closed greenhouse. Transgenic melon and non-transgenic melon plants were cultivated in pots filled with unsterilized soil. After cultivation, the number of microbes, bacteria, actinomycetes and fungi, in the soil was determined. The number of actinomycetes and fungi in the soil cultivated with transgenic melon was slightly larger than the soil cultivated with non-transgenic melon plants. However these differences were not significant, we concluded that the effect of transgenic melon plants on microflora was not different from non-transgenic melon plants. 3. *Agrobacterium tumefaciens* strain LBA 4404 was used as a vector for production of the transgenic melon. Residue of this bacterium on/in plant body was examined in a closed greenhouse. Microorganisms were isolated by shaking or homogenizing the transgenic melon and non-transgenic melon plants in sterile distilled water and plated on YEB media containing certain selective antibiotics. Strain LBA 4404 of *A. tumefaciens* was not detected on the surface or in tissues of the plants. From these results, it is suggested that there are no differences between transgenic melon and non-transgenic melon for harmful impact to other plants and soil microflora. Moreover strain LBA 4404 was not detected on the surface or in tissues of the plants. In conclusion, together with the previous study, the influence of a transgenic melon on the environment was not different from that of a non-transgenic melon within the experiments of a closed and a semi-closed greenhouse. In addition, experiments and cultivation of transgenic melon plants were safely carried out throughout the experiments in

the a closed and a semi- closed greenhouse.

Lecoq, H., Ravelonandro, M., Wipfscheibel, C., Monsion, M., Raccach, B. and Dunez, J. 1993. Aphid Transmission of a Non-Aphid-Transmissible Strain of Zucchini Yellow Mosaic Potyvirus from Transgenic Plants Expressing the Capsid Protein of Plum Pox Potyvirus. *Mol Plant-Microbe Interact* **6**: 403-406.

Transgenic *Nicotiana benthamiana* plants expressing the coat protein of an aphid-transmissible strain of plum pox potyvirus (PPV) were infected by a non-aphid-transmissible strain of zucchini yellow mosaic potyvirus (ZYMV-NAT) in which the coat protein has a D-T-G amino acid triplet instead of the D-A-G triplet essential for aphid transmission. The aphid vector *Myzus persicae* could acquire and transmit ZYMV-NAT from these plants but not from infected *N. benthamiana* control plants that were not transformed or that were transformed but not expressing the PPV coat protein. The aphid-transmitted ZYMV subcultures were shown still to be non-aphid-transmissible from plants not expressing PPV coat protein, which indicated that their transmission was not due to RNA recombination or to reversion to the aphid-transmissible type. In immunosorbent electron microscopy experiments using the decoration technique, virus particles in the infected control plants could be coated only with ZYMV antibodies, while virus particles in the infected transgenic plants expressing the PPV coat protein could be coated not only with ZYMV antibodies but also in part with PPV antibodies. This suggests that aphid transmission of ZYMV-NAT occurred through heterologous encapsidation. These results indicate a potential risk of releasing genetically engineered plants into the environment.

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.

The ability to increase crop disease resistance by using transgenic (TG) means has recently been demonstrated for several crops. The current TG procedures alter the temporal expression of transgene pathogenesis-related (PR) proteins, so that the usually inducible PR proteins are expressed constitutively in the foreign host. The constitutive expression of the transgene PR protein chitinase is believed to increase the host's nonspecific basic resistance to pathogens. A potential nontarget effect of constitutively expressing chitinase may be a decrease in the activity of beneficial microbes, especially vesicular-arbuscular mycorrhizal fungi. The decrease in activity of mycorrhizal fungi is related to reduced susceptibility of TG plant roots to colonization by these fungi, which is in turn associated with lysis of fungal cell walls by the constitutively expressed chitinase. An argument is presented that use of TG means to alter the temporal expression of PR proteins ignores a legacy of past evolutionary trade-offs in vascular plants. A major nontarget effect of expressing transgene chitinase is a reduction in the susceptibility of roots to colonization by mycorrhizal fungi. This reduction in mycorrhizal susceptibility occurs without alteration of the mycorrhizal dependence of the host on symbiont-supplied nutrients. Data are presented in support of this contention that demonstrate a strong negative association between host pathogen resistance and mycorrhizal colonization. An ecological consequence of reducing mycorrhizal colonization is a decrease in the soil's mycorrhizal propagule reserve that diminishes the next crop's production, especially under low- input cropping practices. A further consequence that has both ecological and evolutionary outcomes is the escape of the transgene for improved pathogen resistance into wild populations. By increasing a crop's disease resistance by TG means, we may inadvertently be creating a 'super weed' when the TG plant or the transgene escapes into wild relatives through hybridization. Hybridization of wild relatives with TG plants would be especially relevant for crops, such as sugar beet, rapeseed, and many modern cereal cultivars that have close relatives in the wild but have a relatively low requirement for symbiont supplied nutrients or are nondependent.

Bolton, H., Fredrickson, J. K., Bentjen, S. A., Workman, D. J., Li, S. W. and Thomas, J. M. 1991. Field Calibration of Soil-Core Microcosms - Fate of a Genetically Altered Rhizobacterium. *Microb Ecol* **21**: 163-173.

Microcosms containing intact soil-cores are a potential tool for assessing the risks of the release of genetically engineered microorganisms (GEMs) to the environment. Before microcosms become a standard assessment tool, however, they must first be calibrated to ensure that they adequately simulate key parameters in the field. Four systems were compared: intact soil-core microcosms located in the laboratory at ambient temperature and in a growth chamber with temperature fluctuations that simulated average conditions in the field, field lysimeters, and field plots. These four systems were inoculated with rifampicin-resistant *Pseudomonas* sp. and planted to winter wheat. Populations of the *Pseudomonas* sp. in soil decreased more rapidly at ambient temperature, but population size at the three-leaf stage of wheat growth was the same in all four systems. Populations of the *Pseudomonas* sp. on the rhizoplane of wheat were the same at the three-leaf stage in all four systems, and colonization with depth at the final boot stage-sampling was also similar. In general, microcosms incubated at ambient temperature in the laboratory or in the growth

chamber were similar to those in the field with respect to survival of and colonization of the rhizoplane by the introduced *Pseudomonas* sp.

Bolton, H., Fredrickson, J. K., Thomas, J. M., Li, S. W., Workman, D. J., Bentjen, S. A. and Smith, J. L. 1991. Field Calibration of Soil-Core Microcosms - Ecosystem Structural and Functional Comparisons. *Microb Ecol* **21**: 175-189.

Microcosms containing intact soil-cores are a potential biotechnology risk assessment tool for assessing the ecological effects of genetically engineered microorganisms before they are released to the field; however, microcosms must first be calibrated to ensure that they adequately simulate key field parameters. Soil-core microcosms were compared with the field in terms of ecological response to the introduction of a large inoculum of a rifampicin-resistant rhizobacterium, *Pseudomonas* sp. RC1. RC1 was inoculated into intact soil-core microcosms incubated in the laboratory at ambient temperature (22-degrees-C) and in a growth chamber with temperature fluctuations that mimicked average field values, as well as into field lysimeters and plots. The effect of the introduced bacterium on ecosystem structure, including wheat rhizoplane populations of total and fluorescent pseudomonads, total heterotrophic bacteria, and the diversity of total heterotrophic bacteria, was determined. Fluorescent pseudomonads were present on the rhizoplane in significantly lower numbers in soil inoculated with RC1, in both microcosms and the field. Conditions for microbial growth appeared to be most favorable in the growth chamber microcosm, as evidenced by higher populations of heterotrophs and a greater species diversity on the rhizoplane at the three-leaf stage of wheat growth. Ecosystem functional parameters, as determined by soil dehydrogenase activity, plant biomass production, and N-15-fertilizer uptake by wheat, were different in the four systems. The stimulation of soil dehydrogenase activity by the addition of alfalfa was greater in the microcosms than in the field. In general, growth chamber microcosms, which simulated average field temperatures, were better predictors of field behavior than microcosms incubated continuously at 22-degrees-C.

Wood, M. 1991. Biological Aspects of Soil Protection. *Soil Use Manage* **7**: 130-136.

Soil provides an environment for all forms of life, from viruses and bacteria to trees, and these soil inhabitants play many important roles in soil development and in the maintenance of soil fertility. Policies for protection of soil should therefore include criteria for the biological quality of soil. The establishment of such criteria can only be based on an understanding of the function of the different groups of soil organisms, including the interactions between these groups which give rise to such processes as mineralization of organic matter. However, we are only beginning to understand this most complex of ecosystems. Current information is reviewed and the problems of establishing biological standards for soil are considered. Much of the discussion on assessment of pesticide side effects is relevant to this topic, and the future need to quantify risk associated with the introduction of genetically- engineered organisms into soils is likely to stimulate further debate on this topic.



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