Research Project : ecology of transgenic crop plants expressing insecticidal Bt -endotoxins : effects on trophic interactions and biodiversity of insect pollinators, non-target herbivores and natural enemies

RESEARCH PROJECT

Ecology of transgenic crop plants expressing insecticidal Bt ∂-endotoxins - effects on trophic interactions and biodiversity of insect pollinators, non-target herbivores and natural enemies

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Summary

1 The common soil bacterium *Bacillus thuringiensis* (Bt) is widely used as a biopesticide in agriculture and forest management. In recent years, genes coding for the expression of insecticidal proteins in Bt have been transferred into the genomes of several crop plants by means of genetic engineering. The resulting Bt lines are able to autonomously produce toxins in order to kill relevant herbivore species.

2 Based on previous tritrophic laboratory trials that showed negative effects on non-target organisms (e.g. the economically important predator *Chrysoperla carnea*), concerns have been raised regarding the environmental safety of transgenic Bt crop plants. They include biodiversity issues as well as the maintenance of functions in agro-ecosystems, like pollination and biological control of pests.

3 The EU project Bt-BioNoTa will study non-target effects of different transgenic crop plants (maize, oilseed rape, potato, eggplant) expressing various Bt toxins through multidisciplinary cooperation of five European research institutions. As part of the project, we will investigate possible effects on diversity as well as bi- and tritrophic interactions between transgenic plants, pollinators, non-target herbivores, and their predacious natural enemies in laboratory and field experiments.

4 We will conduct greenhouse feeding experiments with selected plant-pollinator, plant-herbivore, and plant-herbivore-predator systems on transgenic and non-transgenic varieties. Life history parameters will be analysed on an individual basis. Differences in insect species diversity between Bt and non-Bt crops will be studied in field trials in Italy and Hungary.

5 The set-up of the project meets the increasing demand for a better understanding of the environmental impacts of insect resistant transgenic plants. Its outcome will contribute to the establishment of EU policies and regulations for approval and use of Bt crops in Europe.

Keywords: agro-ecosystems, *Bacillus thuringiensis,* biodiversity, non-target effects, transgenic plants, tritrophic interactions

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Introduction

BACILLUS THURINGIENSIS AS AN INSECTICIDE

Efforts to develop effective and environmentally friendly methods of plant protection have lead to the production of highly specific insecticides for use in agricultural systems. These substances aim at saving non-target organisms, limiting the amount and duration of pesticide application, and therefore, reducing negative effects on soils and ground water. Most prominent among these methods is the utilisation of *Bacillus thuringiensis* Berliner (Bt), a gram-positive, rod-like, and spore forming soil bacterium (Krieg 1961; Entwistle 1993; Schnepf *et al.* 1998). During the stationary phase of its growth cycle, Bt synthesizes proteinaceous, parasporal crystals (Cry proteins) inside the cell body (Crickmore *et al.* 1998), which are toxic to a wide range of insect groups (Madigan *et al.* 1999). In susceptible organisms, Cry proteins (∂-endotoxins) are proteolytically converted upon ingestion. The resulting active form binds to specific receptors of the midgut epithelium and induces ion channels or pores in its cells, eventually causing severe tissue damage and gut lysis. Affected individuals die from starvation combined with septicemia when gut contents and toxins infiltrate the body cave (Gill *et al.* 1992).

To date, more than two hundred different types of Cry proteins have been characterized from several strains of the bacterium (Crickmore *et al.* 2001). Each of them has more or less fatal effects on certain herbivore species, mainly lepidopterous, coleopterous, and dipterous insects (Schuler et al. 1998). Thus, foliar insecticides containing Bt formulations have been widely used to control insect pests in agriculture, forestry, and human and livestock health affairs (disease vector control) for almost half a century (Crook & Jarrett 1991).

Since the development of genetic engineering methods in plant production, genes of Bt encoding for the expression of ∂-endotoxins in the bacterium have been transferred into the genome of various crop plants (tobacco, maize, cotton, potato and others) making them extensively resistant against herbivores. With commercial availability starting in the mid 90es, Bt crop plants have taken in significant shares of the seed market and cover an increasing area of arable farm land. In 2000, 44.2 million hectares, of which 99% are located in the United States, Argentina, Canada, and China, were planted with transgenic crops (James 2000). Their growing importance especially in North America has strengthened efforts to introduce Bt crops in other parts of the world as well (Gould 1998). In Europe, applications for commercial release of Bt varieties of several crop plants are pending.

MULTITROPHIC INTERACTIONS

The study of multitrophic interactions, i.e. the interplay of members of different trophic levels (e.g. plants, herbivores and their natural enemies) has become a keystone issue in modern ecological research (see Gange & Brown 1997 for a review). This holds true from both a theoretical and an applied perspective. On the one hand, studying multitrophic interactions can provide insight into basic mechanisms of ecosystem functioning by stressing single chains of the food web. Intensively studied aspects are the effects of plant secondary compounds on higher trophic levels in terms of defence, communication, and population dynamics between the components (Schoonhoven *et al.* 1998; Olff *et al.* 1999). Moreover, a better understanding of interactions between organisms of different trophic levels, e.g. interactions between pests and their natural enemies in agricultural land-

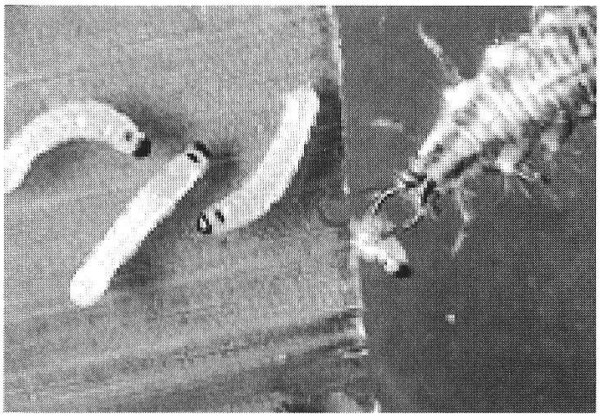

Fig. 1. Chrysoperla carnea *larva feeding on early instar larvae of* Spodoptera littoralis.

scapes, may improve their utilisation for human benefits (DeBach & Rosen 1991). Research on the biological control of pests as part of integrated management systems has demonstrated the economic importance of many parasitoid and predator species, e.g. the green lacewing *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) in Europe and North America (Fig. 1). Larvae of this insect are commercially marketed for biocontrol purposes. They do not only prey on aphids, but also on many other arthropods from eggs to mature stages (Tauber *et al.* 2000).

EFFECTS OF BT TRANSGENES ON MULTITROPHIC INTERACTIONS AND BIODIVERSITY

According to repeated statements from seed producing companies and some scientists, transgenic crop plants expressing ∂-endotoxins of Bt origin are environmentally friendly, i.e. they specifically kill the susceptible target pests and do not affect other organisms in any way. Standardized test protocols employed by industry for the approval of biotechnology products use only a few non-target species. While taking into account lethal effects, they completely neglect less drastic effects on population dynamics which may still affect species diversity in the long run.

Much of the belief in the specificity of Bt is derived from earlier experiments with conventional Bt sprays (Flexner *et al.* 1986; Melin & Cozzi 1989). Here, Bt toxins stay on the surface of the plants only for a short period before being washed away by rain or degraded by UV radiation. Bt plants, however, were developed to produce the toxins continuously in their tissues, so that the latter are much longer available for phytophagous organisms in the plants' environment. Herbivore species that are not susceptible enough to be killed may be exposed to the toxins throughout their entire immature life stage. They may therefore pass the toxins on to species of higher trophic level that prey upon them.

Recent laboratory studies have shown that non-target insects, especially predators like larvae of *Chrysoperla carnea,* can be affected by Bt toxins expressed in plants via the food chain (Hilbeck *et al.* 1998, 1999). When *C. carnea* larvae (Fig. 1) were raised on herbivorous larvae of the non-target moth *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), they exhibited significantly higher mortality and prolonged development if *S. littoralis* had been fed upon Bt maize expressing Cry1Ab toxin compared to the non-Bt treatments. Additional experiments indicated the potential of Bt plants to alter the behaviour, in terms of prey or host selection, not only of *C. carnea* larvae (Meier & Hilbeck 2001a), but also, via the performance of the herbivores, of parasitoid wasps (Schuler *et al.* 1999a). A review of potential side effects of insect resistant transgenic plants on arthropod natural enemies was presented by Schuler *et al.* (1999b).

Only recently, reports have caused considerable public concern that insect resistant crops may also affect insect communities in adjacent vegetation strips due to dispersal of toxin-containing pollen (Losey *et al.* 1999;

Hansen Jesse & Obrycki 2000), and lead to a controversial discussion among researchers (Shelton & Roush 1999; Hunter 2000). Although other tests with non-target insects have revealed no differences between individuals fed on Bt and those fed on non-Bt lines (Pilcher *et al.* 1997; Wraight *et al.* 2000; Zwahlen *et al.* 2000), there is still a number of unanswered questions regarding the ecological risks of Bt crop plant commercialisation in Europe. Unintended effects of Bt plants on non-target organisms might not only endanger the exploitation of natural enemies of pests as biocontrol agents in agricultural crops, but also threaten the biodiversity of ecosystems. The value of biodiversity for the maintenance of ecosystem functions as well as for a sustainable management of agricultural landscapes is being increasingly recognized (Swift *et al.* 1996). Internationally, concern about potential risks posed by genetically modified living organisms motivated "The Conference of the Parties to the Convention on Biological Diversity" to adopt the "Cartagena Protocol on Biosafety" as a supplementary agreement to the "Convention on Biodiversity".

Aim and question of the project

THE EU-PROJECT BT-BIONOTA

To address the crucial question of environmental safety of transgenic insect resistant crop plants expressing *Bacillus thuringiensis* toxins, the European Commission 5th framework project "Effects and mechanisms of Bt transgenes on biodiversity of non-target insects: pollinators, herbivores and their natural enemies" (Bt-BioNoTa) has been initiated. This is a joint effort of five Research and Development groups in The Netherlands, Germany, Switzerland, Italy, and Hungary (Fig. 2)

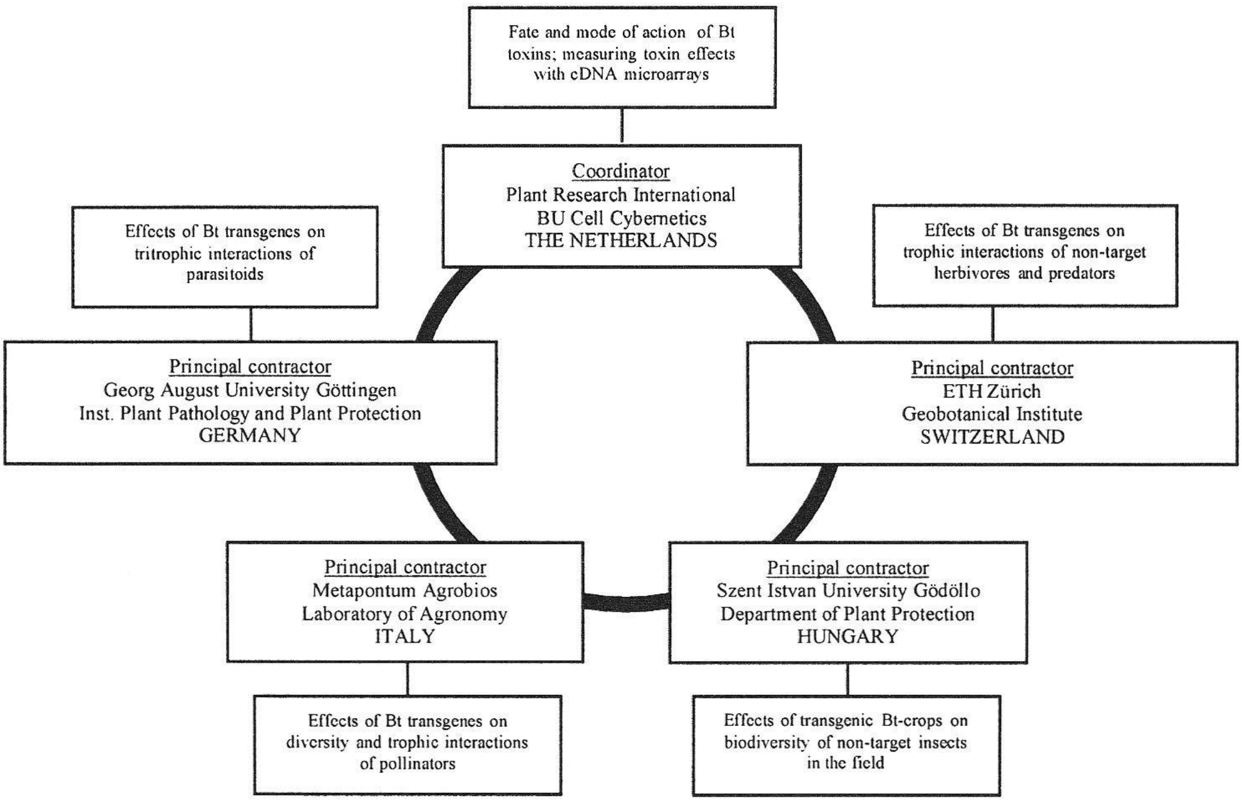

Fig. 2. *Cooperating institutions of the EU-project Bt-BioNoTa and their main responsibilities.*

Table 1. *Crop/Bt-toxin combinations, with their respective target pest to be examined in the scope of the project.*

Crop plant	Bt-toxin	Target pest
maize	Cry1Ab	European corn borer – *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae)
oilseed rape	Cry1Ac	diamondback moth – *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)
potato	Cry1Ab	potato tuber moth – *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)
eggplant	Cry3B	Colorado potato beetle – *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)

to improve our knowledge of non-target effects of transgenic crops on biodiversity by using a multidisciplinary approach and considering different organisational levels (subcellular structure – cell – organism – community – ecosystem) as well as different trophic levels (herbivores, predators, parasitoids, pollinators; Fig. 3).

Laboratory, greenhouse, and field experiments will focus on four transgenic crop

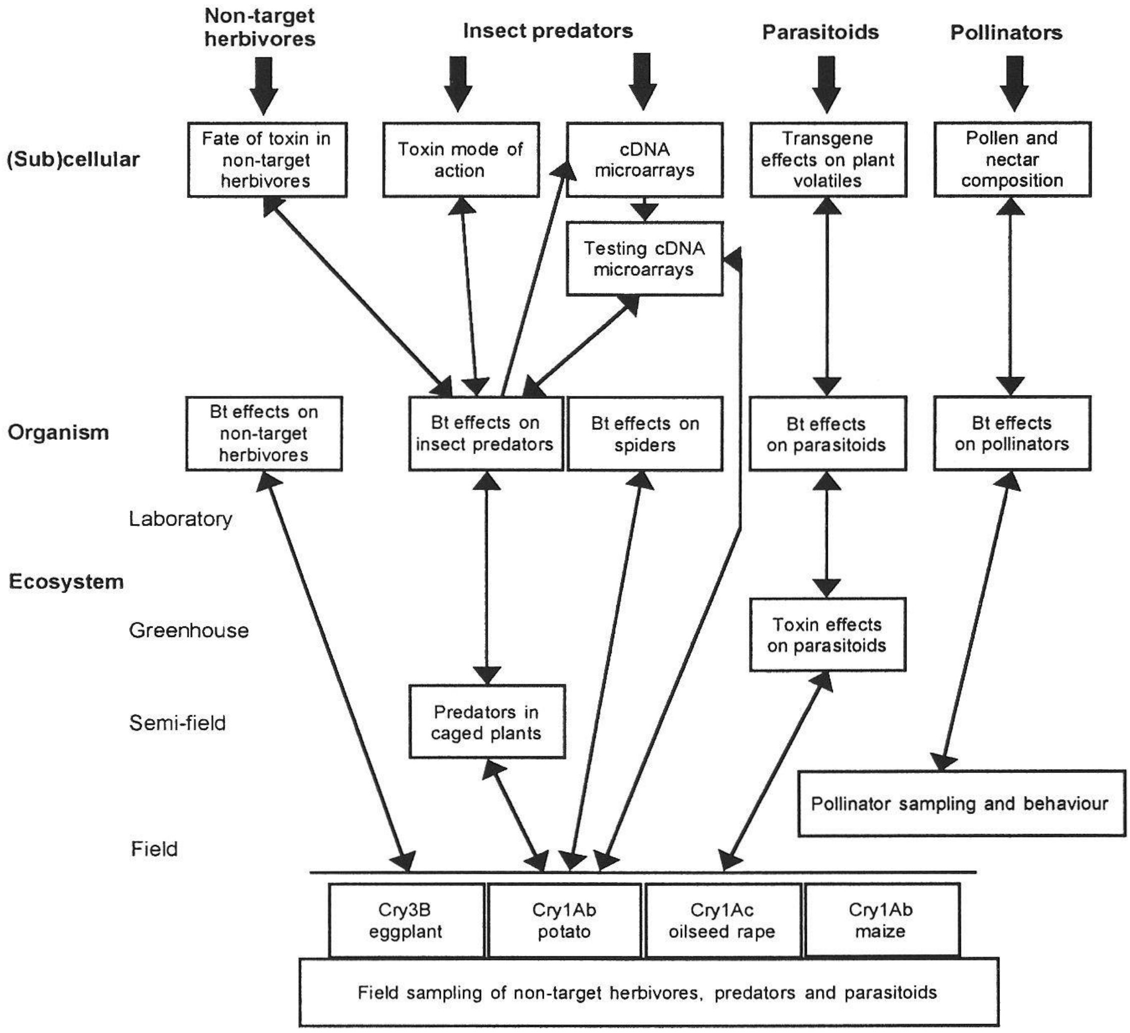

Fig. 3. *The single tasks of the EU project Bt-BioNoTa classified into different organisational levels and trophic groups of the considered organisms.*

plants, maize (corn, *Zea mays* L., Poaceae), oilseed rape (canola, *Brassica napus* L., Brassicaceae), potato (*Solanum tuberosum* L., Solanaceae), and eggplant (aubergine, *Solanum melongena* L., Solanaceae) expressing different Bt-derived Cry toxins against relevant target herbivores (Table 1).

The project will compare Bt and non-Bt lines of the selected crop plants and detect possible effects of lines expressing Bt toxins on non-target arthropods (insects and arachnids). In detail, the project aims at

- elucidating the molecular and genetic basis of the mode of action of Bt endotoxins in the predator *Chrysoperla carnea* and measuring toxic effects by means of cDNA microarrays (Schena *et al.* 1998);
- improving our understanding of effects of transgenic Bt crops on diversity and host finding mechanisms of parasitoid wasps with emphasis on plant volatiles as mediators between trophic levels;
- investigating impacts of transgenic Bt crops on species diversity and community structure of pollinators;
- studying consequences of using transgenic Bt crops for species diversity of predaceous arthropods and tritrophic interactions between transgenic plants, non-target herbivores, and predators;
- examining effects of transgenic Bt crops on species diversity and guild structure of non-target arthropods in the field.

The main responsibilities of each group within the project are outlined in Fig. 2. Field experiments on species diversity issues will be carried out in experimental plots with transgenic Bt crops in Italy and Hungary only.

EXPERIMENTS OF THE ETH GROUP

As a contractor of Bt-BioNoTa our group at the Geobotanical Institute of the ETH Zurich will mainly work on the bi- and tritrophic impacts of transgenic Bt toxin expressing crop plants on pollinators, herbivores, and their predaceous natural enemies. Additional tasks, carried out under the guidance of our cooperation partners, comprise the diversity and performance of pollinators and the molecular mode of action of Bt toxins in the gut of *C. carnea.*

Our first step in conducting multitrophic experiments will be to develop meaningful transgene-herbivore systems for laboratory feeding studies. Suitable phytophagous insects should be associated with the respective crop plant and not be lethally affected by the expressed toxin. Life-history parameters of the employed herbivores will be recorded for the complete larval phase. In addition, longer-term studies will be conducted to involve several consecutive generations of some species to elucidate effects at population level.

Subsequently, we will determine suitable natural enemies of the used herbivores for studying effects on a higher trophic level. Important predators in agricultural systems – and therefore eligible candidates – are lacewings (Neuroptera: Chrysopidae), ladybird beetles (Coleoptera: Coccinellidae), and various groups of spiders (Araneae).

Species diversity and community structure of pollinators will be studied together with our Italian partners in plots of Bt and non-Bt plants of oilseed rape, potato, and eggplant. Moreover, laboratory feeding experiments will be conducted to estimate the effect of pollen from Bt expressing plants on an important pollinator of the Solanaceae family, the bumblebee *Bombus terrestris* L. (Hymenoptera: Apidae).

To investigate mechanisms by which Bt toxins affect the non-target predator *C. carnea,* novel biotechnological methods (cDNA microarrays) will be used by our partners in The Netherlands (Fig. 2). By this means, changes in gene expression of functional DNA sequences of insect cells in response to toxin ingestion can be analysed. To provide test material for these studies, we will conduct feeding experiments with Bt and non-Bt maize plants upon which early instar larvae of the non-target moth *Spodoptera littoralis* are allowed to feed. These larvae will be fed to larvae of *C. carnea* during their entire immature life stage. Shortly before pupation, the *C. carnea* larvae are killed and frozen in order to use them in the molecular experiments. Upon a successful application of cDNA microarrays with *C. carnea,* this method will be extended to other species employed in the feeding experiments.

Research involving transgenic oilseed rape will be conducted in close collaboration with another recently started project at the Institute (Meier & Hilbeck 2001b). There, the main focus will be on gene flow from Bt varieties to wild crucifer species by pollen transfer, and its possible impact on biodiversity of plants and insects.

Relevance of the project

Questions regarding the environmental safety of transgenic plants, not only of varieties bearing insect resistance, have revealed great gaps of knowledge and demand for more research on potential risks involved (Hunter 2000; Wolfenbarger & Phifer 2000). Even in North America, traditionally less critical in this respect, experts have stressed the urgent need to tackle this aspect of novel biotechnological achievements (The Royal Society of Canada 2001; Stewart & Wheaton 2001).

The EU-project Bt-BioNoTa meets some of these requests in an ecological context. By using several combinations of crop plants and Bt toxins as well as a series of chains within the agro-ecosystem food webs, we intend to significantly enlarge the body of knowledge on environmental effects of transgenic insect resistant plants. The multidisciplinary approach will help to achieve the goal and provide urgently needed data. Furthermore, the novel molecular tools employed and evaluated in this study might be valuable in the future as means of monitoring negative effects of Bt transgenes on insects in relatively small samples collected in the field. By using model multitrophic interactions, the planned laboratory feeding experiments will identify potential negative effects on biodiversity that might occur in the field and develop measures to prevent them.

Due to their focus on a limited number of case studies, our experiments will not show whether insect resistant transgenic crops can be considered safe or unsafe in a general way. However, they will enable us to formulate recommendations for future safety studies. These recommendations will support the EU decision-making process and contribute to the establishment of policies, regulations and protocols for the release, approval and use of transgenic, insect resistant crops in Europe.

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