Effects of Bt transgenes on herbivorous insect-parasitoid interactions

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Für Tobias, Carsten und Frank!!!

A lie gets halfway around the world
before the truth has a chance
to get its pants on.

Sir Winston Churchill

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Introduction

Recent newer scientific advances in cell and molecular biology contributed to new technologies in plant breeding culminating in the genetic engineering or modification of crops (Conner *et al.*, 2003). One of those new traits is a built-in gene of *Bacillus thuringiensis* (Bt) in plant genomes responsible for producing the delta endotoxin from this insect pathogen. Thus, the modified plants have the power to produce the toxin protein in its tissues and consequently, killing feeding insects and protecting the plant (Pedigo, 1999). The *Bacillus thuringiensis* toxins causing gut paralysis in respective host larva are used widely and successfully as spray formulations against several Lepidoptera and Coleoptera species in biological pest management. With the background of good degrees of efficiency and specificity of this, the Bt crops were developed to enhance the use for more serious pests which are known to have difficulty in their control (e.g., European corn borer, *Ostrinia nubilalis,* in maize). Therefore, these genetic engineered plants have the potential to overcome the old problems of pest control strategies and may contribute to a reduction of insecticides which were used only as a preventive measure against these pests (Gianessi & Carpenter, 1999; Wolfenbarger & Phifer, 2000). Since introduction of modified Bt crops in the United States, farmers have adopted these new varieties during a few recent years. The reasons for this rapid adoption are primary benefits of increasing yields due to elimination of losses by European corn borer (e. g.) (Carpenter & Gianessi, 2001). Other benefits of modified plants were emphasized by several authors like reduced environmental impact of insecticides, potential of higher yields and better food supply in the developing countries, better food safety due to reduced fungal infections and remediation of polluted soils (e.g., Borlaug, 2000; Mackey & Santerre, 2000; Munkvold & Hellmich, 2000; Mendelsohn *et al.*, 2003; Kasha, 2000; Wambugu, 1999; Zhu *et al.*, 1999).

However, a controversial debate was sparked by the pros and cons of this new technology and is still going on even after 12 years of research. Besides these numerous promises and prospects of Bt transgenes there are still doubts and concerns about potential impacts of these on the environment, human health and food safety in the European public. The public perceptions of biotechnology reveal a widespread growing opposition to genetically modified food during the last few years, whereas the public attitudes to medical applications remain almost positive (Gaskell *et al.*, 2000).

The central question of the public which led to these concerns was: Are Bt crops safe (Corner *et al*., 2003; Mendelsohn *et al.*, 2003)? The legislature and regulatory authorities of the countries around the globe reacted unceremoniously to these concerns of the public. The EU released and adopted the Directive 2001/18/EC in October 2002 which presented a substantially revised version of previous directives. The precautionary principle based on the Cartagena protocol on Biosafety was included as a guide in this directive requiring the evaluation of indirect or delayed effects and changes in agricultural practices (Nap *et al.*, 2003). Thus, the deliberate release into the environment of genetic modified organism (GMO) can only be permitted by the individual Member State of the EU if an environmental risk assessment (e.r.a.) was carried out and revealed no influence of GMOs on human health or environment and further, after release a monitoring plan for the crops is stipulated.

However, the new modified crops could not be the panacea for solving all the pest problems due to specific mode of actions of toxins against the target pests (Sharma *et al.*, 2000). Likewise the questions whether the Bt toxin will have an impact on the environment, the pest management problems that could arise with Bt crops are also an hot spot in the GMO debate. Thus, secondary pests may not be controlled in the absence of sprays which were used for the pest management and therefore, additional sprays or biological control measures will be needed. For instance, the cotton aphids, *Aphis gossypii*, which were once suppressed by early season sprays of endosulfan for *Helicoverpa* spp. in Australia, may be more troublesome in transgenic cotton due to the absence of this insecticidal spray (Herron *et al.*, 2000). Moreover, the Bt maize varieties developed to suppress infestations of the European corn borer, *Ostrinia nubilalis*, are less susceptible to other Lepidoptera pests like *Helicoverpa* spp. which results in a survival of larvae and possibly to a resistance evolution (Lynch *et al.*, 1999; Storer *et al.*, 2003; Tabashnik *et al.*, 2003). Several other problems were also discussed like the migration of polyphagous pests reducing the effectiveness of transgenic crops, economic advantages for farmer and patent rights of GMOs (e. g., Batie & Ervin, 2001; Benbrook, 2001; Dutfield, 1999; Gari, 1999; Horner *et al.*, 2003; IFOAM, 2002; Sharma *et al.*, 2000). However, these topics are discussed and reviewed elsewhere, but the above mentioned problems of Bt and pest management reflect that the expected advantages of Bt crops are not so obvious as they seem to be.

However, the question “Are Bt crops safe” can be answered by identifying potential impacts on the environment carried out with an environmental risk assessment and monitoring of Bt crops as demanded by the EU. This evaluation of potential effects can be conducted on non-target arthropods which are already used in assessments of new insecticidal sprays in the environmental toxicology tests. Non-target arthropods like predators and parasitoids play a crucial role in checking insect-pest populations by helping to prevent some insects from attaining pest status or reducing the damage potential of significant pests (Pedigo, 1999). Therefore, an impact on these species has a direct consequence on the pest population resulting in a possible pest resurgence or replacement (Ripper, 1956). Thus, the query arises why should Bt crops expressing specific toxins to their herbivorous pest species have an impact on natural enemies if a direct threat as expected by insecticidal sprays is not likely? The Bt toxins may or may not be digested by proteolytic enzymes in the digestive tract in non-target herbivores resulting possibly in still active toxins affecting entomophagous natural enemies (Groot & Dicke, 2002). Further, plants play an important role in mediating in a suite of behavioural and physiological interactions amongst the herbivores feeding on them and natural enemies of the herbivores (Harvey *et al.*, 2003). For instances, plants produce a range of toxic secondary compounds which can act as feeding deterrents or significantly alter the physiology and development of herbivores by reduced rates of growth, smaller adult size and increased mortality (van Dam *et al.*, 2000; Giamoustaris & Mithen, 1995; Klun *et al.*, 1967; Niemeyer *et al.*,1989). Moreover, these plant toxins may be sequestered in the haemolymph or body tissue of herbivores resulting in indirect effects on natural enemies by delayed development, reduced hatching rates and low performance (Duffey *et al.*, 1986; Fuentes-Contreras *et al.*, 1998; Harvey *et al.*, 2003; Hunter, 2003). Therefore, we assumed the hypothesis that due to the genetic modification the Bt plants may alter the plant metabolism resulting in adverse effects on parasitoids which develop within host eggs or larvae and have therefore special demands regarding their host quality.

Moreover, parasitoids need highly specific host-plant-herbivore cues which mediate their host location and acceptance (e.g., Lewis *et al.*, 1990; Poppy *et al.*, 1997; Takabayashi *et al.*, 1998; Turlings *et al.*, 1998; Vinson, 1976). Many studies reported about their ability and variability to respond to different host-plant-herbivore cues resulting in acceptance or rejection of the hosts. For instance, it was shown that the specialist parasitic wasp *Cardiochiles nigriceps* was able to discriminate between plants consumed by their host larvae and those consumed by non-hosts although the hosts were presented on phylogenetically different plant species (De Moraes *et al.*, 1998). Further, it was reported that generalist and specialist parasitoids showed preferences for the odours of plant varieties indicating the ability of parasitoids to react to different quantities and qualities of volatile emissions of the plant-herbivore systems (Fritzsche Hoballah *et al.*, 2002; Storeck *et al.*, 2000; Wickremasinghe & van Emden, 1992). Due to these sophisticated host plant-herbivore system, we further assumed that genetically modified plants may emit different volatile blends which possibly lead to a variable attraction of the parasitoids.

The objectives of this study were to investigate the potential impacts of Bt transgenes on different herbivorous insect-parasitoid interactions by behavioural and performance experiments under laboratory and semi-field conditions. Therefore, five herbivorous insect-parasitoid interactions were tested on three different Bt crops (Bt maize, Bt canola, Bt eggplants) with two main questions:

- Do the Bt transgenes influence the parasitoid performance through a bad host quality?

- Do Bt transgenes influence foraging and oviposition behaviour of the parasitoids through a different volatile blend leading to a possible rejection of hosts feeding on Bt plants?

Effects of Bt maize on aphid parasitoid *Aphidius rhopalosiphi*

Abstract

The flight and oviposition behaviour of *Aphidius rhopalosiphi* was investigated to evaluate potential adverse effects of Bt maize expressing CryIAb on parasitoid efficacy. The wind tunnel tests revealed no behavioural changes of female parasitoids. The females did not discriminate between Bt or non-Bt maize-herbivore insect systems. This was also confirmed in dual choice tests in an arena where the response of females was strongly related to uninfested and infested host-plant cues. Parasitization of *Rhopalosiphum padi* did not significantly differ on Bt, isogenic or a conventional maize line. Therefore, we conclude that Bt maize has no adverse effects on this aphid parasitoid with regard to their foraging efficiency and oviposition behaviour.

Keywords: Bt maize, *Aphidius rhopalosiphi*, wind tunnel, arena experiments, tritrophic interactions

Introduction

Public concern about the potential negative impact of transgenic Bt maize varieties expressing Cry1Ab toxin on non-target organisms was fuelled by recent studies on predators and parasitoids reporting higher mortality of these organisms when fed with non-target herbivores which were reared on Bt plants or diet in the laboratory (Chenot & Raffa, 1998; Hafez *et al.*, 1997; Hilbeck *et al.*, 1998 a, b). However, several unsolved questions arose whether these indirect effects on beneficial insects occurred because of a potential effect of Bt-toxin content of the plants on the non-target herbivores, making them less palatable or unacceptable prey or host or whether the feeding tests for the investigated organisms were too artificially designed. Therefore, more researchers conducted additional experiments using a modified experimental set up which resulted in a differentiated view of the previous results. As reported by Raps *et al.* (2001) *Rhopalosiphum padi*, an important host for *Aphidius rhopalosiphi*, ingested or contained no or very low concentrations of CryIAb, whereas in the chewing herbivore *Spodoptera littoralis* CryIAb was detected at concentrations of 4-6 ppb. The studies of Head *et al.* (2001) revealed also no detectable CryIAb in aphids feeding on Bt maize plants. However, Dutton *et al.* (2002) showed that *Spodoptera littoralis* was an unpalatable prey for *Chrysoperla carnea* due to a combined interaction of Bt toxin contents in the larvae and poor prey quality. In addition, Meier & Hilbeck (2002) found in dual choice tests that *Chrysoperla carnea* larvae preferred aphids over Lepidoptera larvae and thus, the findings of previous studies (Hilbeck *et al.*, 1998 a, b) were qualified where *Chrysoperla carnea* larvae were fed with *Spodoptera littoralis* in no choice experiments. Hence, the design of the experimental set up have to mimic the environmental conditions of interaction between the involved organisms.

However, studies on the effect of Bt maize plants on aphid parasitoids have not been done to an extent as has been done for predators. There hasn't been enough evidence as to what extent the Bt maize will have adverse effects on *Aphidius* spp. which are an important natural enemies for aphids on this crop.

The aim of our study was to evaluate the potential effects of Bt maize plants on the aphid parasitoid *Aphidius rhopalosiphi*. We examined the behaviour of *A. rhopalosiphi* in wind tunnel, arena dual choice tests and parasitization experiments with regard to changes in foraging and oviposition behaviour. The tests were carried out under semi-field conditions and represent a realistic background for addressing the question of potential non-target effects of Bt transgenes.

Materials and Methods

Plants

Two maize cultivars Bt-MON810 (Monsanto) and Bt11 (Novartis) maize, expressing *Bacillus thuringiensis* toxin Cry1Ab and their respective non-Bt varieties were used. A conventional maize hybrid (Pedro) was additionally used to test the inherent “background noise” of parasitoid behaviour on conventional accessions of maize hybrids. The maize plants were grown under greenhouse conditions with

1,0 g N/per plant fertilizer during its whole growth period. The maize plants used for the experiments were at growing stage BBCH 30 (stem elongation), because our previous studies with aphids on these plants revealed a strong influence of the younger maize plants on aphid development. In these studies a high mortality of aphids was observed during early growth stages compared to older growth stages of maize plants. Fuentes-Contreras *et al*. (1998) reported about the effects of DIMBOA glucoside in wheat seedlings on the parasitoid *Aphidius rhopalosiphi* where the development time of the parasitoids was increased in such varieties with a higher concentration of DIMBOA. The DIMBOA in maize is also known to inhibit the larval development of *Ostrinia nubilalis* (Klun *et al.*, 1967). Due to this DIMBOA problem, all experiments were adapted to the requirements of the aphids to prevent an uncontrolled mortality on the maize plants. Thus, the aphids were transferred on the maize plants after the beginning of stem elongation.

Insects

The bird-cherry oat aphid *Rhopalosiphum padi* was used as a host for the parasitoid *Aphidius rhopalosiphi*, known to be specialized on cereal aphid species (Braimah & van Emden, 1994). A permanent culture of the aphid species was maintained on a conventional maize hybrid called Magister in a climatic room at 21± 2°C, 70% relative humidity and a photoperiod of 16L: 8D.

Aphidius rhopalosiphi mummies were obtained from Katz Biotech Services, Welzheim. The mummies were maintained under controlled conditions in a climatic chamber until emergence of parasitoid adults. The newly hatched parasitoids of both sexes were placed in a cage without any plant or host material and were provided with a honey solution soaked on dental cotton plugs. The 2-3 days old mated females were used in the experiments.

Wind tunnel

Alighting experiments were conducted using a wind tunnel system (1000 x 600 x 1200 mm) as described by Elst *et al.* (1991). The experiments were set up as dual choice giving parasitoids the opportunity to decide between two different types of host plant-herbivore systems.

The aphids were transferred to maize plants at the beginning of BBCH 31-39 (stem elongation) and reared under controlled conditions during the following 5 weeks.

All plants (up to 2,30 m high) were cut into half 8 hours before starting the tests. To meet the requirements of the wind tunnel system only the lower half of the maize plants were used. An experiment lasted not longer than 3 days ensuring that the plants do not wilt during experiments. The female parasitoids were allowed to gain oviposition experience by releasing them in a cage with conventional maize hybrid treated as described above, before conducting the wind tunnel experiments. Thereafter, the parasitoids were separately caged in gelatine capsules and kept there for another 45 min.

The mated and experienced females were released on a platform downwind to gain volatile cues in the wind tunnel at a distance of 30 cm from the plants and the landings were recorded. During these experiments the wind speed was adjusted to 0,30 m/s and the temperature was maintained at about 22 ± 1°C, 60 ± 10% relative humidity.

If the landings ended directly on one of the two plants within 12 min after release, this flight was recorded as a choice for that plant. If the parasitoids didn’t show any direct flight or any response within the 12 min testing period, this was recorded as no response. Each parasitoid was provided with fresh plants in the wind tunnel.

Arena experiments

We used arena experiments for evaluating the behaviour of the parasitoids if there is any impact of the Bt-plants and their isogenic lines using a small-scale unit in an observation chamber. The dual choice experiments were performed in petri dishes (Ø 9 cm) with an acrylic glass lid. A small hole in the center of the lid provided an inlet for female parasitoids. The bottom of the arena was covered with a white filter paper, which was replaced by a new one in successive tests. The arena was enclosed in a U-shaped frame covered with white cardboard to prevent sunlight and other possible disturbing influences.

The mated females were transferred to a cage with a conventional maize hybrid plant infested with *Rhopalosiphum padi*. The parasitoids were given oviposition experience 45 minutes prior to the dual choice tests. Thereafter, the parasitoids were separately caged in gelatine capsules and kept there for another 15 minutes. Single females were released into the inlet. In the first set of dual choice tests, aphids were offered on white filter paper to the female parasitoids. During the second set of dual choice tests the aphids were again offered on white filter paper; however, two maize leaf disks were additionally placed in the arena. In the third set of dual choice tests the two different maize lines (Bt-MON810 and isogenic line) were placed on the bottom of the arena as 1 x 2 cm leaf disks infested with 5 aphids each and placed 4 cm apart. In all trials a single female parasitoid was observed for 20 min and behavioural responses were recorded.

Each female parasitoid was provided with new leaf disks (1 x 2 cm) in the arena. Further, from these experiments the attacked aphids were maintained and reared under controlled conditions in a climatic chamber for the next 3 weeks to control mummified aphids and adult emergence.

Parasitization test

The parasitization experiment was conducted under semi-field conditions in the greenhouse. A cage of tubular steel scaffolding (266,5 x 120 x 220 cm) covered with a tent made of light-transmissive curtain fabric was erected to cover infested maize plants which were placed within the cage. The tent was pegged on the bottom to prevent female parasitoids from escape. For the experiment eight plants of each variety were infested with three adult aphids and kept to multiply for the next two weeks to a number of 100-150 L2-3 aphids/plant/variety ensuring a total number of 950 aphids on 8 maize plants of each variety.

Before releasing parasitoids, the mated females were transferred to a cage containing conventional maize hybrid plants infested with *Rhopalosiphum padi,* 3 hours prior to the experiment for providing oviposition experience. Thereafter, the parasitoids were collected and introduced into the parasitization cage. After 5 days all aphids were collected and frozen at –80°C to keep them for dissection.

Statistical analysis

The effects of Bt transgenes on behaviour of the parasitoids in the dual choice tests were analysed by using a Chi-square test with the hypothesis H_0= Parasitoids do not prefer any host plant-herbivore complex. No response observations were included in the analysis as an inherent feature of parasitoid behaviour. The dual choice experiments in the arena and the parasitization test were qualitatively analysed by defining the parasitization rate as a successful action resulting in parasitized mummies. Further, these tests were quantitatively analysed by recording the oviposition attacks to the aphids. A Chi-square test with Bonferroni correction (Rice 1989) was applied, because the data were used for more than one test. The parasitization rate was estimated by dissecting the collected aphids giving a total number of dissected aphids of 3710 from all maize varieties. The parasitization test was analysed with the Kruskal-Wallis test, whereas the parasitization of *Rhopalosiphum padi* of the arena experiments were analysed by using the Mann-Whitney U test. These nonparametric tests were carried out with SYSTAT® Version 10 for Windows®.

Results

Wind tunnel experiments

Dual choice tests: Bt11/isoBt11/Pedro

No significant differences were found within the dual choice tests (Fig. 1). The parasitoid flights were randomly distributed between either Bt11 plants as compared to isoBt11 plants or Pedro plants compared to Bt11 plants and did not result in any significant differences. However, when Bt11 plants were compared with Bt11 plants a significant difference occurred due to the high number of no-choice flights (Fig. 1a). Although landings on Pedro plants compared to isoBt11 plants (Fig. 1b) did take place more often, this difference was not statistically significant ($P > 0,05$).

Dual choice tests: MON810/isoMON/Pedro

However, using MON810 plants in the dual choice tests gave a completely different result. Nearly all dual choice tests were significantly different to the hypothesis of random landings ($P < 0,01$), due to the high number of no choice flights (Fig. 2). The response of the parasitoids to the maize variety remained below the expected response and did not differ significantly from other pairs of maize plants tested (Fig. 2a). Moreover, the dual choice test using MON810 vs. the conventional maize hybrid Pedro resulted in a significant difference ($P < 0,01$), due to high number of no choice flights.

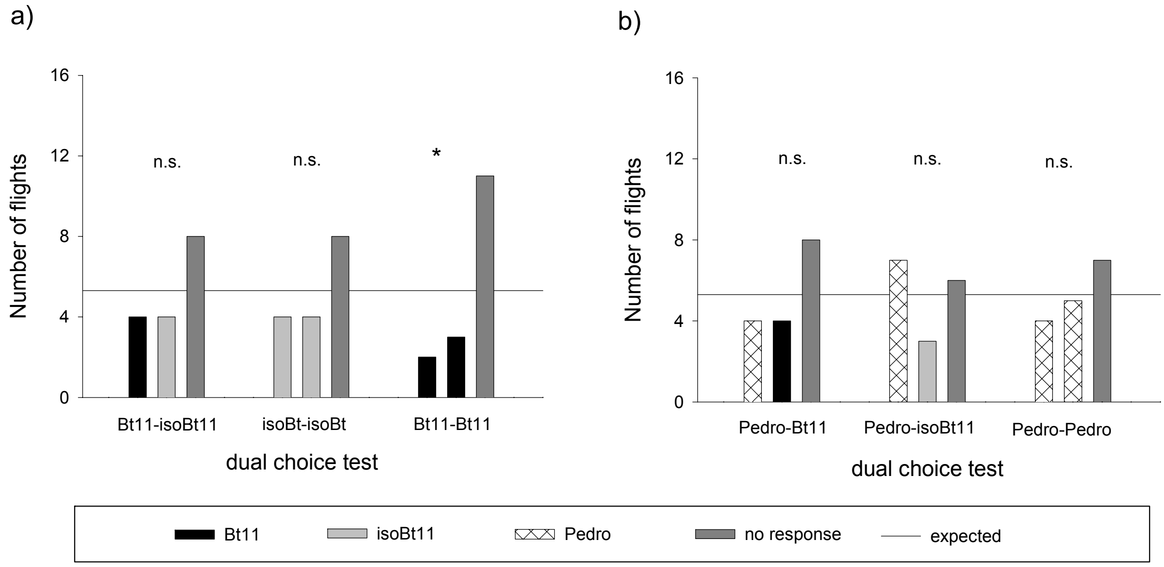

Fig. 1. The response of *Aphidius rhopalosiphi* to host-infested a) Bt11 maize plants and the isogenic line and b) conventional maize hybrid Pedro in a wind tunnel. Number of landings on a plant or no choices were recorded (n= 16). Asterisks indicate significant differences to H_0 (*P < 0,05; **P < 0,01).

No differences were found when comparing the landings on either MON810 plants or Pedro plants (Fig. 2b).

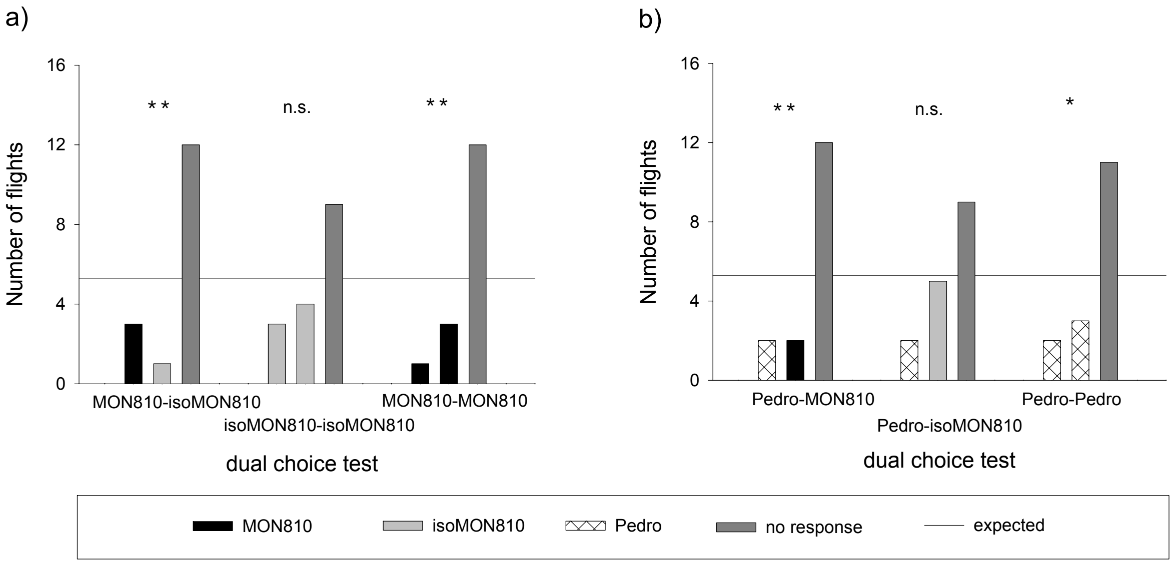

Fig. 2. The response of *Aphidius rhopalosiphi* to host-infested a) MON810 maize plants and the control isoMON810 and b) conventional maize hybrid Pedro in a wind tunnel. Number of landings on a plant or no choices were recorded (n= 16). Asterisks indicate significant differences to H_0 (*P < 0,05; **P < 0,01).

Arena experiments

No significant differences were found when analysing the dual choice tests in the arena (Fig. 3). The frequencies of responses of the female parasitoids did not differ from the expected ones ($P > 0,30$). The four choice categories were nearly uniformly distributed.

As an important feature of the behaviour of female parasitoids the choice category for both, aphids on Bt and isogenic leaf disks, was included, emphasizing the ability of parasitoids to respond to the offered aphids in all possible ways like accepting or rejecting one host or the other.

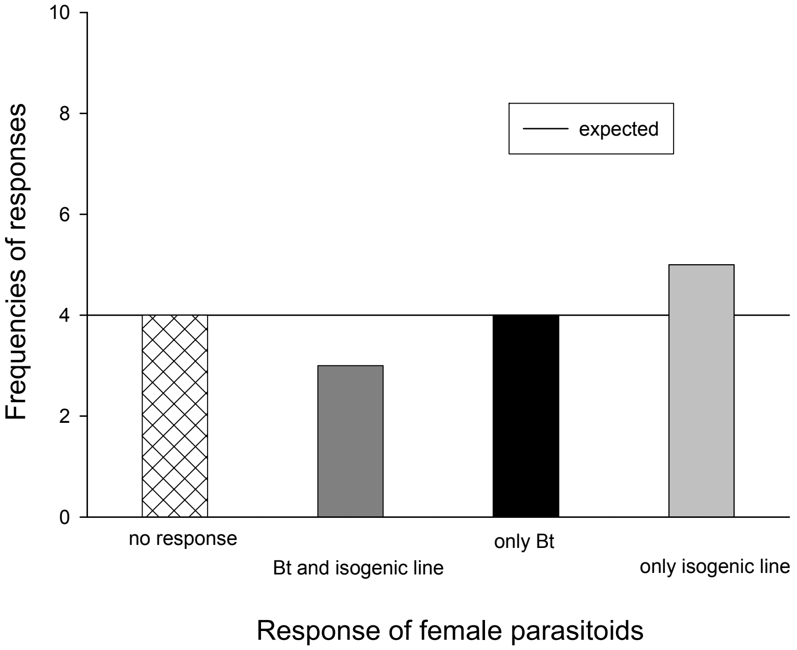

Fig. 3. Response frequencies of *Aphidius rhopalosiphi* to host-infested Bt maize and isogenic leaf disks (MON810) in dual choice tests in an arena. H_o: The response of female parasitoids is uniformly distributed. (Kolmogorov-Smirnov test; $D_{max}= 0,438$; $P > 0,30$).

Moreover, the number of aphids attacked on Bt leaf disks were not significantly different compared to aphids on isogenic leaf disks (Table 1).

Although the parasitization rates were different in aphids feeding on Bt MON810 plants (37,9 %) as compared to aphids feeding on isogenic MON810 leaf disks (43,3 %) these differences were not statistically significant (Pearson Chi-square= 0,075; df=1; $P > 0,50$).

Tab. 1: Parasitization of *Rhopalosiphum padi* in dual choice test in the arena

	Total number of attacked aphids (N=160)	Total number of mummies	Adults hatched	
			Males	Females
MON810	29[1]	11	6	5
isoMON810	30[1]	13	8	3

[1] Mann-Whitney U-test statistic=121,00; *P* > 0,50.

Furthermore, due to weak responses of *A. rhopalosiphi* in previous dual choice tests in the arena the experimental set up was changed in an additional experiment.

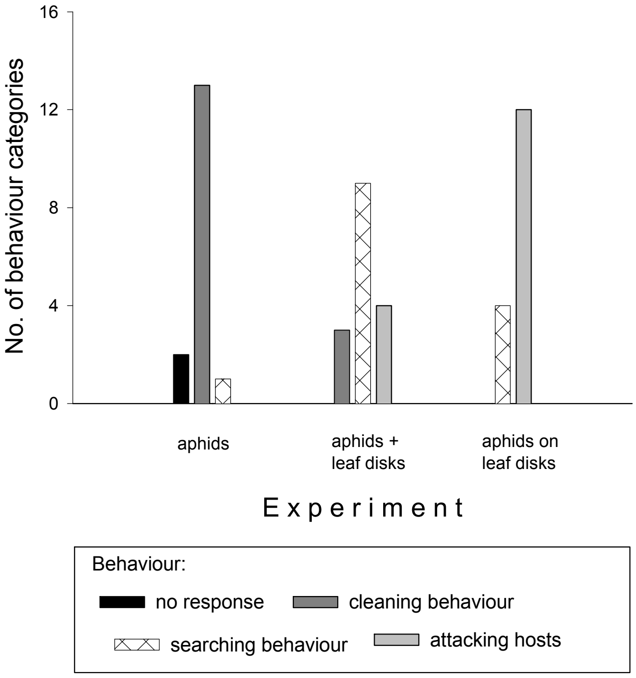

Fig. 4. Implications of arena tests with different experimental set ups on the behaviour of *Aphidius rhopalosiphi*. (Chi-square test; sequential Bonferroni correction; *P* < 0,05).

More female parasitoids were attacking aphids when offered on leaf disks compared to aphids on filter papers alone or aphids provided with pure volatiles of the leaf disks (Fig. 4). The differences between searching and attack behaviour was significantly higher in the experiment aphids on leaf disks compared to the behaviour in the experiment aphids and leaf disks (0,05 < *P* > 0,02).

Parasitization experiment

The dissection of the collected aphids per plant variety resulted in a parasitization rate of 8 % on Bt-MON810 as compared to 12% on the conventional plants and 10,5% on isoMON810 plants (Fig. 5). The parasitization rate per plant and plant variety did not differ significantly (*P* > 0,50).

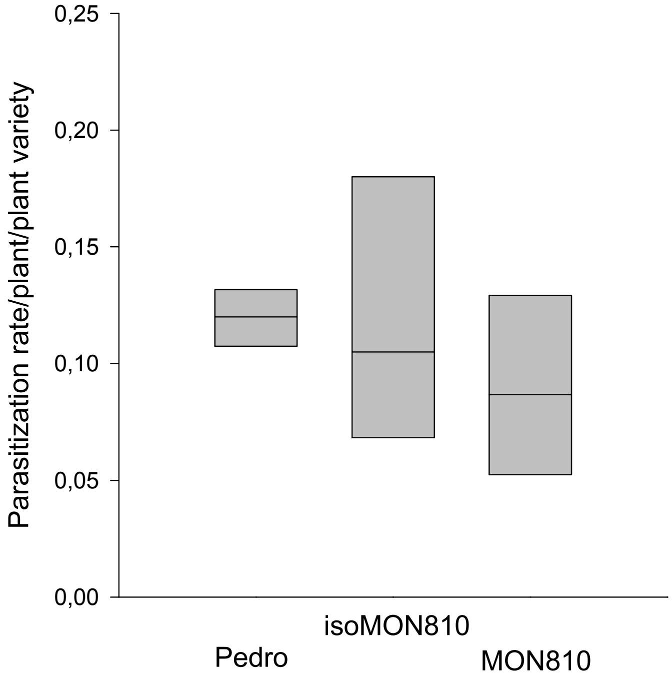

Fig. 5. Parasitization rate of *Rhopalosiphum padi* on MON810, the isogenic line and a conventional hybrid Pedro. Boxes and whiskers represent quartiles and ranges, respectively. Outlier are shown as dots (Kruskal-Wallis test= 1,254; df= 2; *P* > 0,50).

Discussion

The problems of an uncontrolled mortality of the herbivores in younger maize plants related to the effects of DIMBOA glucoside reflect that not only due to expression of the Bt toxins a different metabolism can be expected, but also to the complex physiological responses of the maize plants during different growing stages. Moreover, we conclude from these problems that the effects of the Bt toxin expression are not measurable during the early growing stages, because it is not clear whether the DIMBOA glucosides will interfere with Bt toxin expression or not. Furthermore, a testing system can not exclude these additional effects resulting in misleading data if not the younger growing stages of maize plants are avoided in the experiments.

We found no evidence for any influence of a Bt-toxin expression in transgenic plants on the foraging efficiency of aphid parasitoids. The wind tunnel experiments did not result in a preference for a specific host plant-herbivore system due to the different maize hybrids tested. Although *Aphidius rhopalosiphi* is able to discriminate between different wheat varieties (Wickremasinghe & van Emden, 1992), yet the parasitoids in our experiments neither discriminated between a Bt plant-host-complex as compared to the isogenic line nor between a Bt plant in relation to a conventional maize hybrid. Thus, the null hypothesis could not be rejected. Although we found significant differences due to a high number of no choice flights as compared to choice flights, landings on each plant variety were almost equally high and close to a 50/50 ratio. Consequently, the parasitoids were not much influenced by different maize lines.

The high number of no choice flights can be explained by a typical behaviour of *Aphidius rhopalosiphi* which is more sensitive and susceptible to environmental changes and effects than other aphid parasitoids like *Diaeretiella rapae* or *Ephedrus plagiator*. However, due to a susceptible behaviour female parasitoids are put faster under stress in new situations than a non susceptible behaviour and thus, a count for the no choice category is consequently been made if the parasitoids didn’t show any direct flight. Furthermore, it was observed that the number of no choice responses can be reduced with parasitoids who had a previous experience in the wind tunnel.

Therefore, the high number of no choice responses is also a matter of associative learning of the parasitoids.

The results of the arena experiments supported the findings in the wind tunnel tests. The females did not discriminate between aphids sucking on leaf disks of the different varieties. Moreover, the number of aphids attacked was nearly equally high and did not result in different numbers of mummies. However, the female biased sex ratio of parasitoids emerging from mummies of the isogenic leaf disks differed as compared to the sex ratio of adults emerging from mummies of the Bt leaf disks. This difference would have been due to an effect of prolonged maintaining subculture of *A. rhopalosiphi* at Katz Biotech Services; we do not relate these differences to be an effect of the varieties tested.

Furthermore, additional experimental set ups of the arena tests revealed which factors of volatile stimuli were important for the female parasitoids. The female parasitoids started to search intensively only when uninfested maize leaf disks were introduced into the arena. The visual and volatile cues of the maize leaf disks had a triggering effect on the parasitoids. The searching behaviour response decreased when infested maize leaf disks were introduced. The females started immediately an attacking behaviour towards the aphids whereas the attacking behaviour in the latter test could only be observed when an aphid started to settle and suck during the tests on the leaf disks. This is regarded as evidence for the attractants of pure maize volatiles resulting in a searching behaviour of the parasitoids and also regarded as evidence that the attacking behaviour of the parasitoids started only in the presence of aphids. Braimah & van Emden (1994) were also able to demonstrate that *Aphidius rhopalosiphi* attacked more aphids on leaf disks than on filter paper and addressed the importance of host-plant cues for this parasitoid.

A further conclusion of these behavioural observations is that females of *A. rhopalosiphi* discriminate between pure volatiles and volatiles emanating from host-infested plants, as it has been shown in several herbivore-parasitoid interactions in recent years (e.g., Guerrieri *et al.*, 1993; Micha *et al.*, 2000; Mölck *et al.*, 1999).

We did not find significant differences while analysing the parasitization rates on the maize varieties in the parasitization experiment. This finding corroborates the previously discussed conclusion of a non specific plant-host choice of the female parasitoids. The low parasitization rate could have been caused by the high aphid densities per plants during this experiment which did not allow the parasitoids to gain a successful oviposition.

This inverse density-dependent parasitism by *A. rhopalosiphi* was observed also in our laboratory culture where the parasitization rate could not be increased by enhancing number of hosts per plant.

In conclusion, we found no evidence that female parasitoids of *Aphidius rhopalosiphi* discriminated between plant-herbivore stimuli of Bt maize plants compared to controls plants. Moreover, we did not observe any adverse effects of Bt-varieties on oviposition behaviour of the parasitoids. These findings confirm the conclusion by Raps *et al.* (2001) who assumed that due to the absence of CryIAb toxins in aphid hosts harm to natural enemies is unlikely to exist.

The behaviour of the females of *A. rhopalosiphi* was strongly related to the volatile cues emanating from uninfested and infested maize plants. Thus, host plant-herbivore stimuli did affect the behaviour more than factors potentially related to the engineering of the new plant trait.

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Effects of Bt canola on aphid parasitoid *Diaeretiella rapae*

Abstract

Dual choice tests in the wind tunnel and parasitization experiments under semi-field conditions were carried out to evaluate potential adverse effects of Bt canola expressing CryIAc on the aphid parasitoid *Diaeretiella rapae*. The flight response of the females revealed no behavioural differences towards Bt and non-Bt canola herbivorous insect systems. Aphid densities did not influence the choice of the parasitoids regarding the different canola varieties. Cues induced by herbivores feeding on the host plants were more important than specific cues for searching females irrespective of the transgenic or non-transgenic line. Adult emergence rate did not differ significantly between Bt and non-Bt canola. We conclude that the foraging efficiency and oviposition behaviour of this parasitoid species is not significantly influenced by Bt canola plants and expression of CryIAc had no detrimental effects on the development of the parasitoid larvae.

Keywords: Bt canola, *Diaeretiella rapae*, wind tunnel, transgenic plants, tritrophic interactions

Introduction

Although insect-resistant transgenic canola lines have not been commercialized in Europe yet, the impact on non-target organisms of these transgenic engineered lines were investigated in several studies (Girard *et al.*, 1998; Potting *et al.*, 1999; Rahbé *et al.*, 2003; Schuler *et. al.*, 1999, 2003; Stewart *et al.*, 1996; Winterer & Bergelson, 2001). In these studies the discussion centered around potential effects of transgenic canola on behavioural responses of herbivores feeding on these plants and their parasitoids and predators. Rahbé *et al.* (2003) demonstrated that the cysteine protease inhibitor oryzacystatin (OC-I) had a significant influence on aphid species *Myzus persicae*, whereas Schuler *et al.* (2001) found that the Bt toxin Cry1Ac had no detrimental effects on either aphid population or their parasitoids. The latter authors recommended to establish an intermediate level of bioassays resulting in a three–tiered test system of increasing scale, complexity and realism to assess potential risks of these plants. These intermediate level bioassays should be a combination of small-scale laboratory bioassays and large-scale field experiments at a population level consisting of experiments with regard to parasitoid behaviour and performance (Schuler *et al.*, 2000). So far, studies on effects of Bt canola haven't been carried out for *Diaeretiella rapae*, an important natural enemy of the mealy cabbage aphid, combining behavioural tests such as parasitization and dual choice tests with regard to the entire complex behavioural responses of this parasitoid.

D. rapae associated to cruciferous plant-herbivore systems responds also to grass plant-herbivore systems and is highly sensitive to changes in glucosinolates contents and their metabolic products (Bradburne & Mithen, 2000; van Emden, 1978; Vaugh *et al*., 1996).

The aim of this study was to evaluate the effects of Bt and the wild type canola plants on the aphid parasitoid *Diaeretiella rapae*. A set of wind tunnel tests and parasitization experiments were conducted to investigate the potential impacts of Bt canola towards a non-target organism with regard to changes in complex behavioural responses of the parasitoid to the expression of Bt-toxin by these plants.

Materials and Methods

Plants

We used a canola line event Westar 063 of a high expressing Bt line Westar (Stewart *et al.*, 1996), expressing Bt toxin Cry1Ac, which is toxic against several Lepidopteran larvae (Schuler *et al.*, 2001). As a control we used the untransformed canola line of Westar (wild type). These plants were grown in the greenhouse until the growing stage BBCH 30 (beginning of stem elongation) and BBCH 60-67 (beginning – end of flowering). The plants were supported with a 15-15-15 (N: P: K) fertilizer weekly.

Insects

A culture of the mealy cabbage aphid *Brevicoryne brassicae* was maintained on a conventional canola line (Lipocosmos) in a climatic room at 23 ± 1°C, 70% relative humidity and a photoperiod of 16L: 8D.

The parasitoid culture of *Diaeretiella rapae* originated from individuals collected from a canola field near Göttingen were reared on mealy cabbage aphid and maintained also in a climatic room at a temperature of 24 ± 1°C, 60-70% relative humidity and a photoperiod of 16L: 8D. Before start of an experiment every time the mummies were collected from the plants in the parasitoid culture and reared in a climatic chamber until adult emergence. Thereafter, the parasitoids were transferred to cages with an uninfested canola to give parasitoids plant volatile stimuli. Before starting the tests the parasitoids were given experience with an infested plant to herbivory stimuli and herbivores.

Wind tunnel experiments

These experiments were conducted in a wind tunnel system (1000 x 600 x 1200 mm) described in Elst *et al.* (1991). The experiments were set up as dual choice to allow the parasitoid to decide between two different types of plant-herbivore systems. The mated females experienced with plant-herbivore stimuli were released on a platform in the wind tunnel at a distance of 30 – 40 cm from the plants. The plants were artificially infested with *Brevicoryne brassicae* (60 to 200 aphids/plant) depending on the experiments. In dual choice tests comparing Bt vs. wild type canola, plants had densities of 200 aphids. While in tests where different aphid densities and influences of canola lines were compared, plants had densities of 60 and 200 aphids per plants.
If a female parasitoid landed directly on one of the plants within 20 min after release, it was recorded as a choice for that plant. However, if the parasitoids didn't exhibit any direct flight or any response within the 20 min, it was recorded as a rejected choice (no response) for the offered plants. Each female parasitoid was provided with new plants in the wind tunnel.

Parasitization experiments under semi-field conditions

To test whether the Bt canola plants had an influence on parasitization behaviour and efficiency of the parasitoids, two different parasitization experiments were carried out. For the first experiment 12 canola plants of each variety were infested with 3 adults of *Brevicoryne brassicae*; these mother aphids were allowed to multiply until a colony size of 60 L2-3 aphids/plant/variety was achieved. The female parasitoids were released beneath the randomly placed plants in a climatic room and allowed to parasitize the aphids. On 10th day the mummies were counted and collected for further experiments.

The second parasitization experiment investigated the influence of different feeding duration of the herbivores on the parasitoids. This test was performed under semi-field conditions in the greenhouse. For this experiment 12 canola plants of each line were infested with adult aphids as described above. After 14 days another 12 canola plants of each variety were infested with adult aphids and left to multiply to 100 L2-3 aphids/plant/variety. After another 14 days the female parasitoids were released among the randomly placed plants and allowed to parasitize their hosts.

Statistical analysis

The wind tunnel experimental results were analysed by using a Chi-square test with the hypothesis H_0= Parasitoids do not prefer any host plant-herbivore system. Therefore, it was expected that the response of females were uniformly distributed over all three choice categories (Bt canola, iso canola, no response). Moreover, for this analysis the third choice category “no response” representing rejection or no response ability of parasitoids was included as an inherent factor of parasitoid behaviour.

The parasitization rate was calculated as the number of mummies/plant in relation to the total number of aphids per plant. The parasitization data were analysed with the nonparametric Mann-Whitney U test and Kruskal-Wallis test carried out with the program SYSTAT® Version 10 for Windows®. Whereas a Chi-square test with Bonferroni correction (Rice 1989) was implicated in the analysis of the emergence rates, because the data were used for more than one test. The emergence rate was calculated as the number of parasitoids emerged per day in relation to the total number of mummies.

Results

Wind tunnel experiments

Females did not prefer to land on one specific plant. Therefore, we did not find a significant difference to H_0. Moreover, we did not find any significant difference in the dual choice test Bt canola versus isogenic canola, although the number of flights towards the isogenic canola was slightly higher (Fig. 1). A significant difference was observed within the control dual choice experiment Bt canola vs. Bt canola due to the high number of no choice flights ($P < 0,05$). Finally, the control dual choice test isogenic canola vs. isogenic canola did not result in the expected 50/50 ratio of landings.

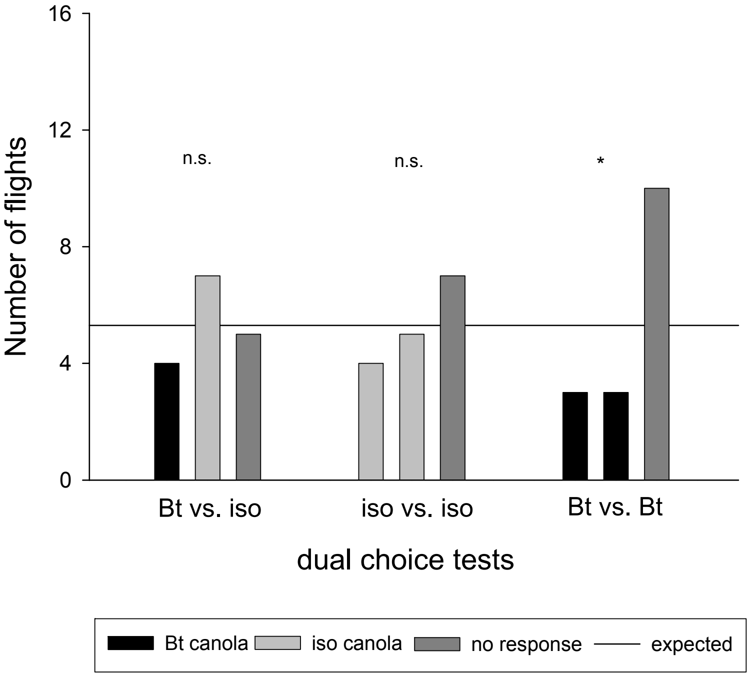

Fig. 1. Response of *Diaeretiella rapae* to Bt canola and isogenic plants in a dual choice test in wind tunnel (n= 16). Asterisks indicate significant differences to H_0 (*$P < 0,05$).

When repeating the wind tunnel tests Bt vs. wild type canola, we increased the number of parasitoids per dual choice test to n= 28. However, again we found no significant difference between parasitoids flight response of the towards Bt canola plants as compared to isogenic canola plants (Chi-square test= 0,55; df= 2; *P* > 0,50).

To test whether the aphid densities had an influence on the behaviour of the parasitoids towards different canola varieties, another wind tunnel test was performed. The comparison of the flight responses did not show a significant difference between the dual choice tests with low (60 aphids per plant) and high (200 aphids per plant) aphid densities (*P* > 0,50). Moreover, the dual choice tests Bt canola vs. isogenic canola using high aphid densities resulted in a flight distribution, which did not differ from random expectation (Fig. 2).

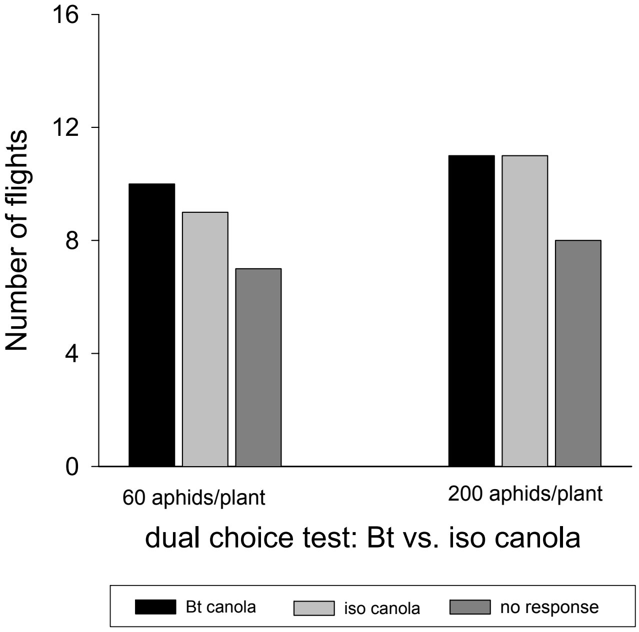

Fig. 2. Flight response of *Diaeretiella rapae* to Bt vs. isogenic canola plants with different aphid densities (Chi-square test= 0,03; df= 2; *P* > 0,50).

Parasitization experiments

During the semi-field parasitization experiments the parasitoids were given a choice to parasitize aphids on the Bt or isogenic canola line. This experiment resulted in a higher interquartile range of 3,3 – 41,7 percent of the parasitized hosts on Bt canola as compared to the isogenic canola with an interquartile range of 0,8 – 14,2 percent of parasitized hosts (Fig. 3). The median of the parasitization rate was 12% and 8% for Bt canola and iso Canola and resulted not in a significant difference (*P* > 0,20). The adult parasitoid sex ratio was female biased with 62,4% and 47,5% on Bt canola and isogenic canola.

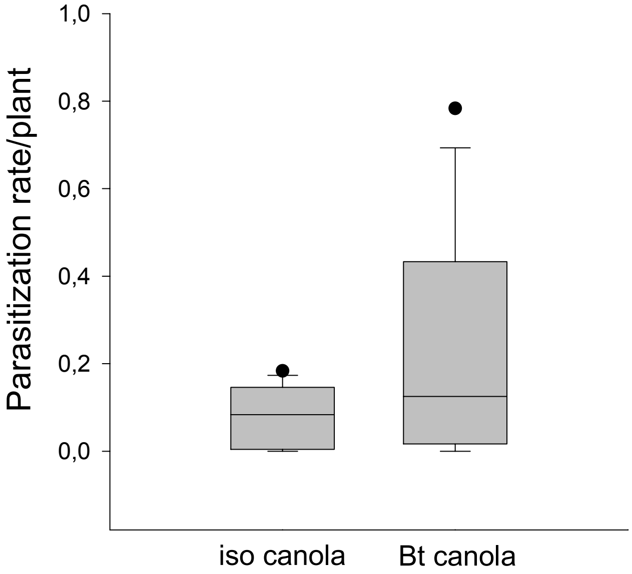

Fig. 3. Parasitization rate of *Brevicoryne brassicae* on Bt canola (Westar) and wild type canola (Mann-Whitney U test= 51,500; *P* > 0,20).

In another parasitization experiment we tested the influence of different feeding intervals of the aphid hosts on parasitoids behaviour.

Interestingly, the parasitization rates of aphid hosts feeding for 2 weeks on canola plants were significantly lower as compared to host feeding duration of 4 weeks on the plants (*P* < 0,002). However, no significant differences were found when comparing the Bt canola varieties and the isogenic lines (Fig. 4). The parasitization rate of aphids feeding for 4 weeks on Bt canola and isogenic canola was twice as high as the parasitization rate of aphids feeding for 2 week on these plants. Furthermore, the adult emergence reared on hosts with different feeding duration significantly differed (*P* < 0,05; Chi-square test, Bonferroni correction).

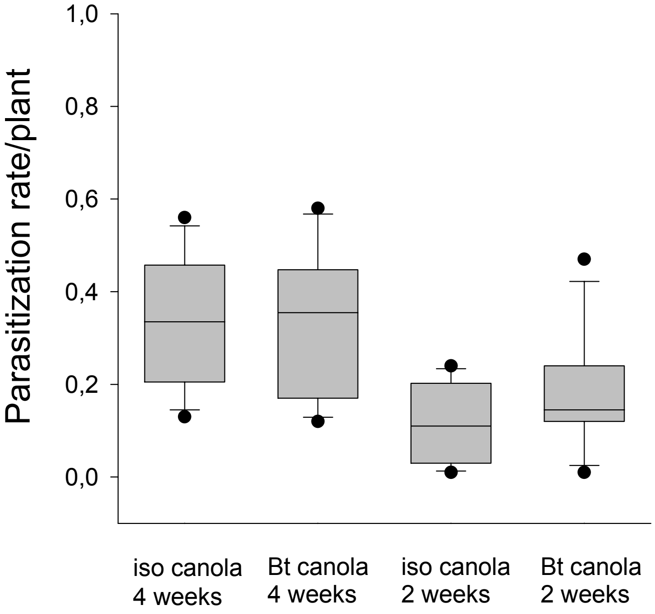

Fig. 4. Parasitization of *Brevicoryne brassicae* with different feeding duration on Bt canola and the wild type. (Kruskal-Wallis test= 16,872; df= 3; *P* < 0,002).

Parasitoids from hosts feeding for 4 weeks on canola emerged on average 0.5 to 1 day in advance as compared to those ones hatching from 2 weeks feeding aphids (Fig. 5).

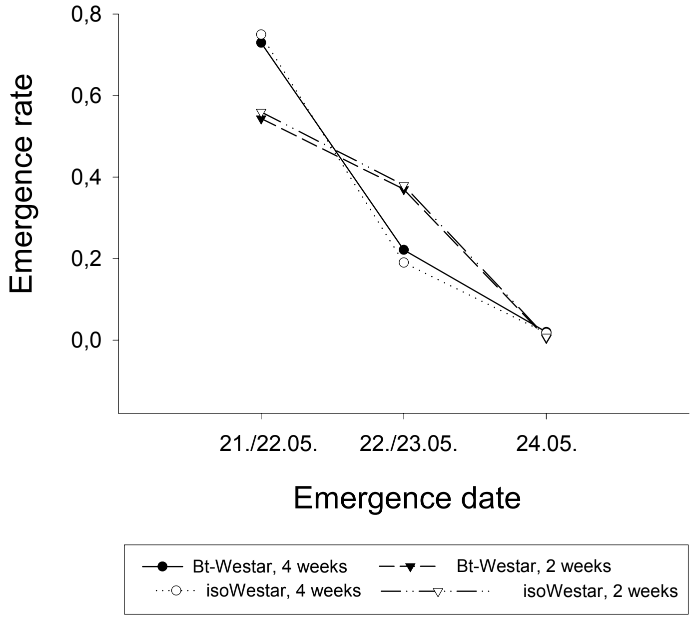

Fig. 5. Emergence of *Diaeretiella rapae* developing in aphids feeding on Bt canola and the wild type. (*P* < 0,05; Pearson-Chi-square test, Bonferroni correction).

Discussion

The flight response of *Diaeretiella rapae* was not influenced by aphids infested on Bt or wild type canola. The females could not distinguish between host plant-herbivore set ups offered in the wind tunnel. Therefore, we did not find a significant difference to H_0 assuming that female parasitoids do not prefer any canola lines. However, due to the relative low number of parasitoids per dual choice tests the flight response in test Bt canola vs. wild type could be interpreted as a more pronounced response for the canola wild type due to a higher number of landings on the latter plants, although these differences were not significant. But further wind tunnel tests with a higher number of parasitoids per dual choice test did not corroborate this hypothesis. In these experiments landings on Bt canola as compared to the wild type did not differ from the expected uniform distribution. Furthermore, the response of the parasitoids was not related or different with regard to the plant varieties when aphid densities per plant were higher. Aphid densities did not influence the choice of the parasitoid female with regard to different canola varieties. Therefore, we conclude that the females did not take into account which plant variety (Bt canola (Westar) vs. wild type) they were searching on. Although *Diaeretiella rapae* is a generalist parasitoid and is able to parasitize many aphid species, it is associated to cruciferous plants and parasitization rates are in general higher on hosts on these plants (Pike *et al.*, 1999; Vaugh *et al*., 1996).

Furthermore, van Emden (1978) reported in a study about the sensitivity of *Diaeretiella rapae* to changes in glucosinolates contents of a new Brussels sprouts variety resulting in rejection of the hosts. Thus, suggesting that the females of *Diaeretiella rapae* are sensitive to changes in the volatile spectrum of crucifers and do display a differential behavioural response when alighting these plants we conclude that Bt canola plants are not essentially different to the wild type and therefore, behavioural responses towards these plants do not differ.

It is still an open question, whether the female parasitoids in this species are able to discriminate minor changes in the quantity and quality of volatile compounds. Subtle differences found in the experiments may be related to these differences. However, each conventional cultivar also differs with regard to volatile profiles emitted, and behavioural responses of the parasitoids are thus not specifically related to transgenic plants.

The parasitization rate of aphids on Bt canola and the wild type did not differ significantly. The female parasitoids were not attracted more to one of the plant-herbivore system offered, assuming that a higher parasitization rate could be related to a host preference for that plant-herbivore system. Therefore, our results support the findings of Schuler *et al.* (2001) who also found no significant differences in aphids feeding on both canola varieties.

Furthermore, the Bt canola variety did not impact the development of the parasitoids. However, in the experiment with differing feeding times the parasitoids responded to plant-host complexes that were infested longer. The females of *Diaeretiella rapae* were attracted more to infested plants which were older as compared to younger infested plants. However, again females did not discriminate between the different varieties. Thus, this is regarded as further evidence for the conclusion that the parasitoids did not discriminate between the different canola varieties tested or were not able to recognize subtle changes in volatile blends. The preference for older infested plants may be explained by a different and more attractive volatile blend (Bradburne & Mithen, 2000) or by enhanced stimuli of typical visual cues (i.e. intensive sucking spots of aphids on the leafs). Moreover, Vater (1971) reported that colour preferences are an important feature for plant recognition in *Diaeretiella rapae*. Sucking spots are much more intensive on older infested leafs as compared to younger infested leafs. Moreover, this preference is corroborated by shorter hatching times found in adults emerged from the Bt canola and wild type of the older infested plants as compared to adults which emerged from the younger infested plants. This temporal shift may be explained by stimuli which were more attractive for the parasitoids on these plants resulting in a resting on these plants. This may have been in advance as compared to other plants and/or longer than visiting and resting on the alternative hosts. A longer resting on a plant-host system has direct consequences for temporal distribution of the parasitoids and their parasitization behaviour. The fact of spending more time (i.e. up to half a day or longer) on a special host plant-herbivore system means that the females will visit alternative hosts later on during the day and that means these hosts will be parasitized later than the first visited hosts. These different parasitization times will be carried through the whole development time resulting in an earlier hatching to adult parasitoids of the first visited hosts.

These studies demonstrated that the parasitoids were not influenced by behavioural responses to aphids feeding on Bt canola as compared to the isogenic lines. Host plants cues were more important for searching females than specific cues (if there were any at all) related to the transgenic line. Moreover, the expression of the Bt-toxin in Westar 063 did not alter the efficiency of the parasitoids when parasitizing the cabbage aphids.

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Effects of Bt eggplants expressing Cry3B on the parasitoid species *Aphidius ervi* and *Encarsia formosa*

Abstract

The aphid and whitefly parasitoids, *Aphidius ervi* and *Encarsia formosa* were examined with regard to behavioural changes and detrimental effects of Bt and non-Bt eggplant host systems on parasitoids under laboratory and semi-field conditions. More aphids were parasitized by *Aphidius ervi* on Bt eggplants; however this could not be explained by a preference for this host plant-herbivorous insect system. However, physiological traits of the two varieties differed at the fruiting stage which resulted in an indirect effect on the herbivore-parasitoid system. Development and adult parasitoid emergence were not influenced by Bt eggplants. Furthermore, the experiments did not show differences in mortality and longevity of the F1 *A. ervi* generation in parasitoids from Bt and non-Bt eggplants. Moreover, parasitization by *Encarsia formosa* was not influenced by Bt or non-Bt eggplants. Finally, the emergence rate of adults of *Encarsia formosa* was not altered by the Bt eggplants.

Keywords: Bt eggplants, *Aphidius ervi*, *Encarsia formosa*, tritrophic interactions

Introduction

Genetic engineered crops open newer possibilities and offer solutions which could be advantageous over conventional of pest management strategies (e.g., Dale *et al.*, 2002; Sharma *et al.*, 2000; Verkerk *et al.*, 1998; Wolfenbarger & Phifer, 2000). For instance, new modified crops expressing the *Bacillus thuringiensis* (Bt) toxins exhibit an in-built protection against the target pests during its growth stage which reduces the cost for insecticides simultaneously increase the yield (Gianessi & Carpenter, 1999). However, such interventions in the natural properties of crop plants could result in unwanted effects on non-target herbivores and their parasitoids and predators (Hansen-Jesse & Obrycki, 2000; Hilbeck *et al.*, 1998; Schuler *et al.*, 1999). A worst case scenario could be a complete shift in plant-herbivore system resulting in an imbalance of herbivory due to differential toxicity for susceptible and resistant target pests. Furthermore, the potential non acute toxic effects on non-target pests could result in resistance to the modified crops and eventually to newer primary and secondary pests. Further, this can result in a non-acceptance of the parasitoid hosts and even worst, an effect on the parasitoid fitness and efficiency. Several studies have been conducted in this direction and to name a few crops like Bt-maize, Bt-canola and Bt-potatoes (e.g., Birch *et al.*, 1999; Hilbeck *et al.*, 1999; Schuler *et al.*, 1999). However, only a few studies have been done on potential impact of genetically modified vegetables concerning interactions between non-target herbivores and their natural enemies.

Bt eggplants expressing Cry3B, a toxin targeted against the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, has been developed and tested in Italy since several years (Arpaia *et al.*, 1997; Iannacone *et. al.*, 1997), and yet, eggplant cultivation normally harbours a complex of insect pests like spider mites, aphids and whiteflies. These insect pests could be normally controlled by using mass produced natural enemies in glasshouse production systems. In some cases this integrated pest management strategy using beneficial insects has been shown to be more successful than the use of insecticides (Ravensberg, 1992; Stary, 1993). Thus, a new trait exhibited by a transgenic vegetable expressing Bt toxin targeted against only one pest species makes another use of these pest management strategies necessary. Therefore, it should be tested whether the genetic engineered vegetables bear a potential to disrupt or convert plant-herbivore-natural enemy interactions to allow adjustment of biocontrol strategies to these altered situations.

The aim of the present study was to evaluate the effect of an eggplant cultivar expressing Cry3B on the interaction between two herbivore species (*Aulacorthum solani* and *Trialeurodes vaporariorum*) and their parasitoid species (*Aphidius ervi* and *Encarsia formosa*). We compared the parasitization rates of hosts to evaluate the ability of the parasitoid species to control these host populations on a transgenic cultivar and the related isoline. Further, emergence rates, longevity and mummification rate of the aphid parasitoid were investigated to evaluate potential adverse effects on their performance and development.

Materials and Methods

Plants

The eggplants expressing the Bt toxin Cry3B and the isogenic line were grown in plastic pots (Ø 13 cm) in composted garden soil under greenhouse conditions until the growing stage BBCH 50-51. During this period, the plants were fertilized weekly with complex fertilizers (nitrogen: phosphate: potassium = 15: 15: 15%).

Insects

Aphidius ervi – Aulacorthum solani

A stock culture of the spotted potato aphid, *Aulacorthum solani* was initiated from individuals of a clone originating from a *Hibiscus sabdariffa* plant. This culture was maintained under laboratory conditions (22 ± 2°C, 70% relative humidity and a photoperiod of 16L: 8D) on a conventional eggplant variety called Ritmo.

Mummies of the parasitoid *Aphidius ervi* an effective enemy for this aphid were requisitted from re-natur company, Ruhwinkel, Germany. These mummies were maintained in petri dishes (Ø 9 cm) under controlled conditions in a climate chamber until hatching of adults. After mating, females of *Aphidius ervi* were released in a cage along with a conventional eggplant variety Ritmo ensuring pest free to assure their contact with the plant structure and the volatiles emitted.

Encarsia formosa – Trialeurodes vaporariorum

A permanent culture of the greenhouse whitefly, *Trialeuordes vaporariorum*, was maintained under laboratory conditions (24 ± 2°C, 70% relative humidity and photoperiod of 16L: 8D) on eggplants variety Ritmo.

Encarsia formosa parasitized nymphs were received from Katz Biotech Services, Welzheim/Germany. These nymphs were also maintained in climate chamber with 24 ± 2°C, 70% relative humidity and photoperiod of 16L: 8D until hatching of adults.

Experimental set up

Aphidius ervi – Aulacorthum solani

Parasitization experiment under semi-field conditions

Twenty-one plants of the Bt- and the isogenic variety were placed randomly in a climatic room prior to aphid infestation. Three adult aphids per plant were transferred to the eggplants with a brush and were allowed to feed on these plants for the next 2 weeks when the number of aphids had multiplied to approximately about 100 L2-3/plant. In a situation were the aphids were more than 100 they were removed to appropriated number. The 2-3 days old female parasitoids were released randomly within the infested plants. On 8th and 10th day after release, number of mummies developed so far, were counted and on day 10 all mummies were harvested from each plant. These mummies were further reared in climate chambers for recording emergence rate and sex ratio.

Longevity test

After emergence, the *Aphidius ervi* adults were sexed as males and females and were divided into two Bt and isogenic eggplant groups. Group (1) was termed as parasitoids emerged on 20./21.07. and group (2) was termed as parasitoids emerged on 21./22.07. Each group, comprising 80 parasitoid individuals with 60% females, were released into a Perspex cylinder (Ø 15 cm and 30 cm high). The Perspex cylinder was lined with a filter paper in the shape to increase the potential surface for resting of the parasitoids. The parasitoids were provided with honey solution applied on dental cotton plugs along with moistened dental cotton plugs. The dental cotton plugs were replaced every second day by new ones. The longevity of the parasitoids was determined from eclosion until the last parasitoid died. The mortality of the parasitoids was recorded daily.

Encarsia formosa – Trialeurodes vaporariorum

Parasitization experiment under laboratory conditions

20 adult whiteflies were caged per eggplant for 48 hours. Eggs laid by whiteflies were counted and only 20 eggs/leaf/plant were left by removing the excess ones. The

larvae were on hatching reared until larval stage L_2-L_3. Twenty infested eggplants of each variety were divided into five groups with four plants per cage and variety. Thereafter, one day old adults of *Encarsia formosa* were introduced into these cages and allowed to parasitize whitefly nymphs for 24 hours. The parasitoids were removed from the plants and these were placed in a separate laboratory room for next 15 days. Parasitized nymphs were collected from the leaf surface in vials for further observation.

Statistical analysis

Mummification rate was determined as the relation of mummies counted at day 8, the beginning of mummification, to the total number of mummies at day 10. Thus, this rate represents larval development of the parasitoids. The parasitization rate per plant of *Aulacorthum solani* was determined by counting the total number of mummies of *Aphidius ervi* on day 10 after releasing the females in relation to the total number of aphids infested per plant. Parasitization rate and mummification rate was analysed by using the Mann-Whitney U test. This test was carried out with the program SYSTAT® Version 10 for Windows®. The adult emergence rate was calculated as the number of parasitoids emerging per day in relation to the total number of mummies. The Chi-square test assuming no differences between adult emergence reared on Bt and isogenic lines. The longevity data of two groups were analysed by comparing daily mortality of parasitoids between these groups using Kruskal-Wallis test.

Further, the parasitization rate per plant of whitefly nymphs was calculated as the number of parasitized nymphs in relation to total number of nymphs infested per plant. The emergence rate of *Encarisa formosa* was calculated as the number of adults emerged per total number of parasitized nymphs. The data were analysed using Mann-Whitney U test.

Results

Aphidius ervi

Parasitization experiment under semi-field conditions

The mummification rate did not differ significantly between the aphids on Bt and isogenic eggplants (*P* > 0,50). The median of the mummification rate for aphids on Bt and isogenic eggplants was nearly identical with 42% mummies/plant on Bt eggplants and 39% mummies/plant on isogenic line (Fig.1).

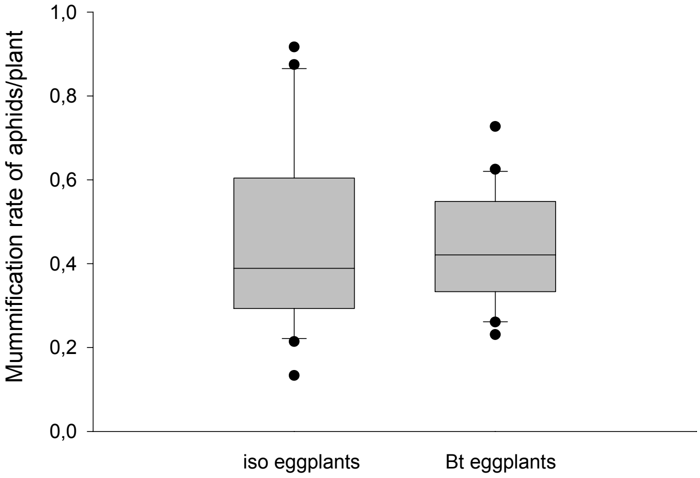

Fig. 1. Mummification of *Aulacorthum solani* on 8th day after parasitization on Bt and isogenic eggplants. Boxes and whiskers represent quartiles and ranges, respectively. Outlier are shown as dots (Mann-Whitney U test= 222,50; *P* > 0,50).

Further, the parasitization rate was significantly different between aphids on Bt eggplants than on the isogenic line (*P* < 0,001). The median of the parasitization rate for aphids on Bt eggplants was 23% per plant, whereas the parasitization rate for aphids on the isogenic line, was 12% per plant (Fig. 2).

The number of adults emerged showed female sex ratio of 55,27% biased on isogenic line and 51,83% on Bt eggplant.

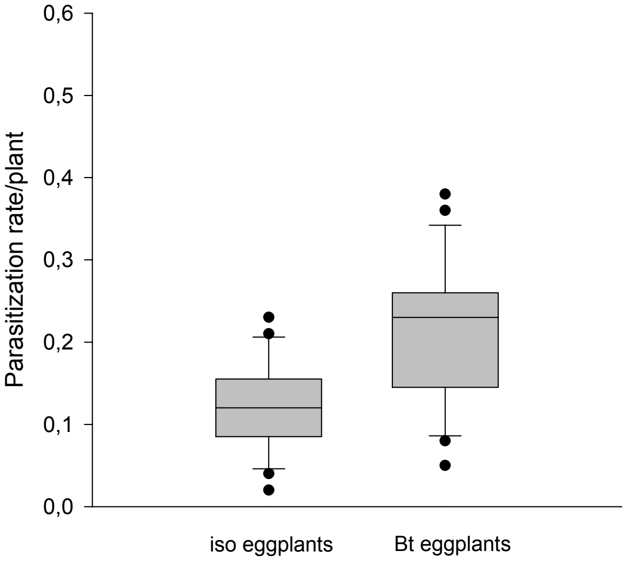

Fig. 2. Parasitization of *Aulacorthum solani* on Bt and isogenic eggplants. Boxes and whiskers represent quartiles and ranges, respectively. Outlier are shown as dots (Mann-Whitney U test= 357,00; *P* < 0,001).

No significant differences were found when analysing emergence of parasitoids reared on aphids from Bt eggplants as compared to aphids from isogenic eggplant (*P* > 0,50). The emergence rate for a period of 6 days was nearly identical between both lines (Fig. 3).

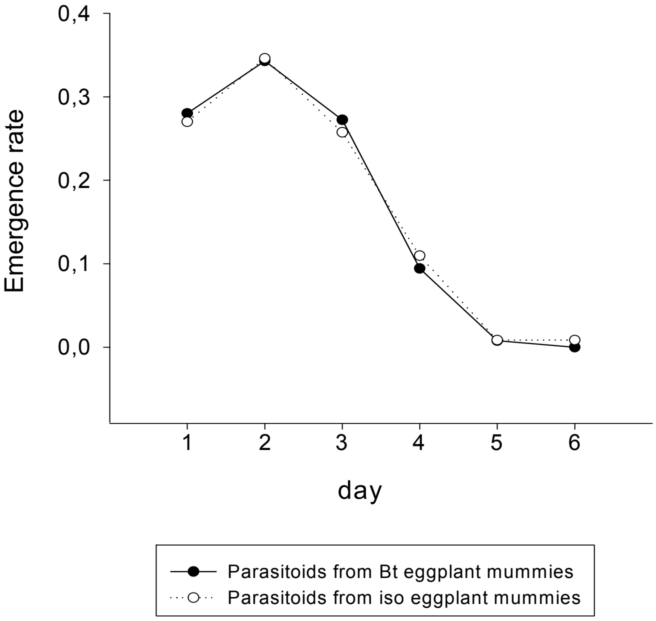

Fig. 3. Emergence of *Aphidius ervi* developed on aphids on Bt eggplants and iso eggplants. (Mann-Whitney U test= 18,500; *P* > 0,50).

Longevity test

Adult longevity of the parasitoids did not differ between Bt eggplant and isogenic lines (0,10 < *P* > 0,05). However, a difference was found between the mortality of parasitoids within the group (1) of Bt and isogenic wasps (Fig. 4).

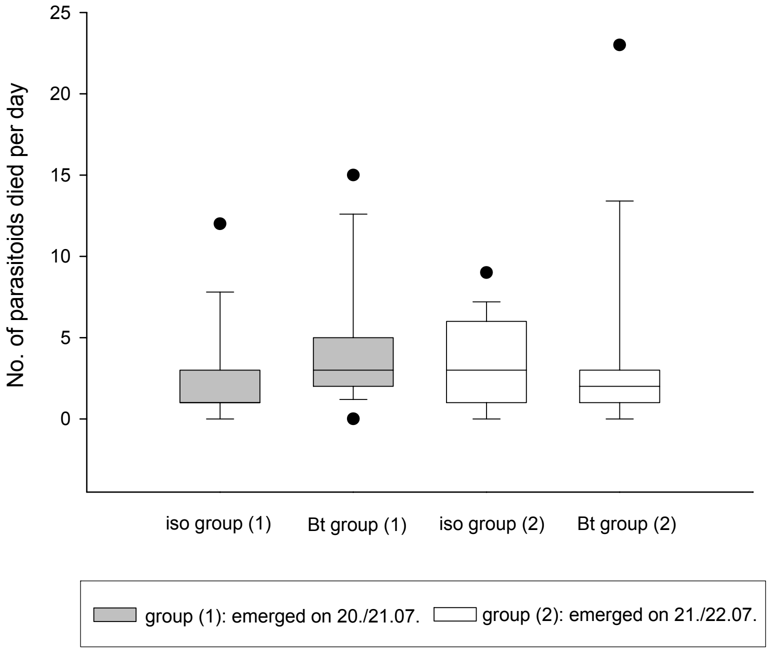

Fig. 4. Mortality of *Aphidius ervi* developed on aphids of Bt and isogenic eggplants. Boxes and whiskers represent quartiles and ranges, respectively. Outlier are shown as dots (Kruskal-Wallis test statistic= 6,402; df= 3; 0,10 < *P* > 0,05).

Encarsia formosa

There was no difference in parasitization rate of nymphs on Bt plants and isogenic eggplants (*P* > 0,20), although the median of parasitization rate of whitefly nymphs was only 27% on isogenic line and 40% on Bt eggplants (Fig. 5).

The emergence rate of *Encarsia* adults reared on nymphs of Bt plants did not differ significantly from those nymphs reared on isogenic plants (*P* > 0,10).

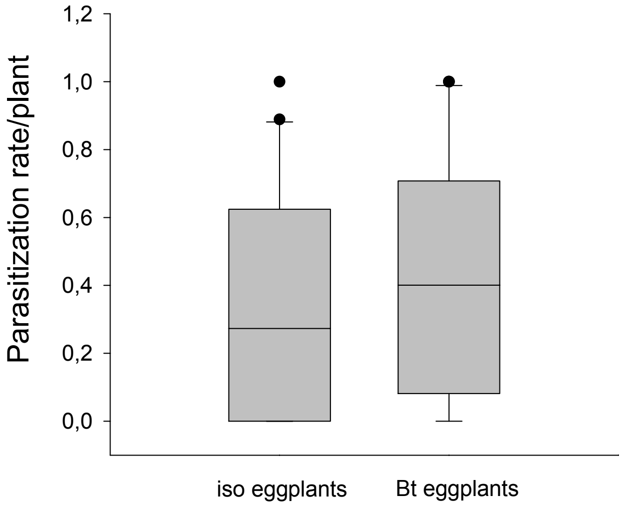

Fig. 5. Parasitization of whitefly nymphs on Bt eggplants and the isogenic line. Boxes and whiskers represent quartiles and ranges, respectively. Outlier are shown as dots (Mann-Whitney U test= 242,500; *P* > 0,20).

The median of the emergence rate of adults reared on isogenic nymphs was 60%, whereas the median of adults reared on Bt nymphs was only 55,7% (Fig. 6).

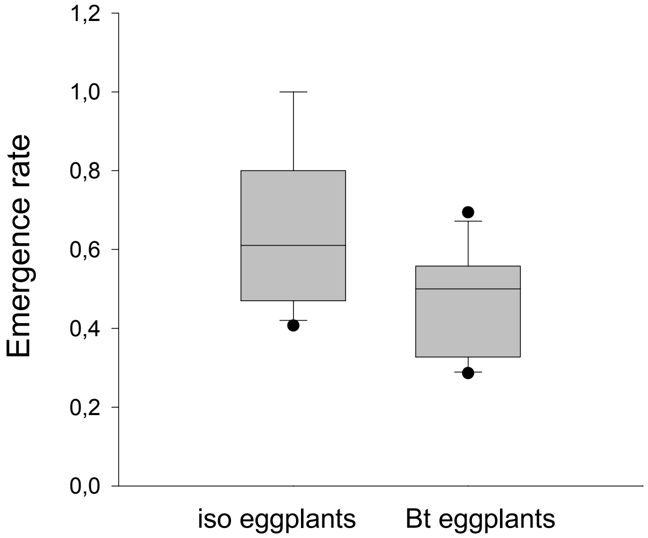

Fig. 6. Emergence rate of *Encarsia formosa* reared on nymphs on Bt and isogenic eggplants. Boxes and whiskers represent quartiles and ranges, respectively. Outlier are shown as dots (Mann-Whitney U test= 115,00; *P* > 0,10).

Discussion

Aphidius ervi

In our experiments a higher parasitization rate of aphids was observed on Bt than on isogenic eggplants. This higher parasitization rate could be due to a preference for herbivorous hosts feeding on the Bt eggplants or some adverse effects of the hosts on larval development of the parasitoids. A host preference result in more visits on these plants, which in turn result in higher parasitization rates of the hosts feeding on these plants. Moreover, parasitoids attracted to a specific host plant-herbivore system do spend more time in searching for their hosts on these plants than on the alternative host plants. As Ives *et al.* (1999) demonstrated the mean time spent by *Aphidius ervi* on such plants after encountering aphids was three times greater than on plants where no aphid was encountered. These authors noticed that a decrease in foraging efficiency was greatest when the average number of aphids per plant was low. Thus, it seemed to be likely that the female parasitoids spent more time on an eggplant due to high aphid density per plant before moving to the plants nearby. Furthermore, spending more time on a host plant than on an alternative host plant has a direct consequence for the life history traits of aphids which will be parasitized. Thus, assuming that duration of larval development of parasitoids is almost fixed under the climatic conditions in our experiments, a delay in parasitization of alternative hosts from 14 to 24 hours including several resting sorties and non-active phases of parasitoids will result in a later beginning of larval development of these parasitoids. Consequently, an early emergence of adult parasitoids could be expected where hosts were longer and/or earlier visited on plants. However, the emergence rate of the parasitoids from the Bt and isogenic eggplants did not differ significantly. Following the above conclusions, we assumed that the females did not spent more time on one of the eggplant varieties resulting a delay in parasitization. This could also be an indication that the female parasitoids were not attracted to a specific host-infested variety. Hence, the effect of this higher parasitization rate on Bt eggplants could not be explained by a clear preference of the parasitoids.

However, the observed differences in the parasitization rate might have been be related to some cultivar properties of the isogenic variety which were different than Bt eggplants. During the parasitization experiment older leaves became yellow and abscised in the isogenic plants. This protracted senescence is an important process in plant physiology which results in nutrient resorption to prevent losses so that the nutrient pool will be translocated towards fruit developing (van Heerwaarden *et al.*, 2003). This physiological process probably would have resulted in shifting of aphid population on the plants. The aphids would have left this yellowing leaf or probably died during this process resulting in a reduced number of aphids per plant on the isogenic line.

As mentioned previously, another reason for the higher parasitization rate could still derived from adverse effects of less suitable hosts for development of parasitoid larvae. These effects would result in a longer larval developmental time or higher mortality of parasitoid larvae during early larval stages. This particular effect could not be confirmed, because we found no significant differences in aphid mummification rate between either on Bt or isogenic eggplants. At beginning of mummification an equal number of parasitoid larvae passed through this larval development stage. Further, adult longevity and daily mortality of the parasitoids did not differ significantly. Parasitoids of Bt eggplants lived as long as the adults from the isogenic eggplants. A difference was found within group (1) where more adults reared on Bt hosts died. This might have been caused by a bad honey solution made of raisins which were possibly contaminated with fungi or agents for food conservation and provided to Bt group (1).

However, Ashouri *et al.* (2001) reported that immature survival and adult size of *Aphidius nigripes* were affected when developing within hosts feeding on transgenic potatoes expressing Cry3A. Further, development of *Aphidius ervi* was not affected by hosts feeding on potatoes expressing a cysteine proteinase inhibitor against parasitic nematodes (Cowgill *et al.*, 2004). Thus, as mentioned by Ashouri *et al.* (2001) different forms of potato resistance affect parasitoids in various effects on their fitness.

In conclusion, our results did not indicate an impact of Bt toxin Cry3B on the parasitoids. Neither oviposition behaviour nor development of the parasitoids was influenced. Hence, the effects of genetic modified plants of Solanacecae depend highly on the specific *B. thuringiensis* δ-endotoxins or other resistance modifications and the plant species. However, still there is an open question if the 2nd generation of parasitoids reared on Bt or isogenic eggplants will show adverse effects for their fitness and efficiency.

Moreover, physiological traits of plant varieties might have caused an indirect effect on the herbivore-parasitoid system. Thus, varietal-dependent effects of plants are not only reflected in Bt or non-Bt toxin expression, but also in physiological demands during different growth stages of the plants.

Encarsia formosa

As Guerrieri (1997) reported the *Encarsia formosa* is affected by chemicals deriving from the host-plant complex and visual cues of these plants regarding the host location from a distance. Whereas, the host searching behaviour on a leaf is random with respect to the presence of hosts (van Roermund & van Lenteren, 1995). There were no adverse effects of Bt eggplants on *Encarsia formosa* parasitizing whitefly nymphs as compared to isogenic eggplants. Several plant factors such as plant species, variety and morphological features can affect the efficiency of *Encarsia* females (Hoddle *et al.*, 1998). We concluded that the females were not influenced differently by these plant factors. The data showed a high interquartile range of parasitized hosts per plants. This could be due to host-feeding behaviour of *Encarsia* avoiding parasitization of hosts which were previously used for feeding (Nell *et al.*, 1976). As van Lenteren *et al.* (1977) reported the whiteflies are controlled by *Encarsia* only on an intermediate level on eggplants. These results are confirmed by our study where on average 30-40% parasitization per plant was achieved on Bt and isogenic eggplants. Furthermore, the adult emergence rate was not significantly different between these two varieties. Consequently, the Bt eggplants had no influence on the development of the *Encarsia*.

In conclusion, the efficiency of *Encarsia formosa* seems not to be affected by Bt or isogenic eggplants. However, still an open question is if the Bt eggplants will have an effect on *Encarsia* efficiency and fitness and thus, further studies will be needed to evaluate these possible impacts.

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Effects of Bt maize on *Helicoverpa armigera* and its consequences on egg parasitoid *Trichogramma brassicae*

Abstract

The Old world bollworm *Helicoverpa armigera*, a serious pest of maize in South and Southeast Europe, and its egg-parasitoid *Trichogramma brassicae* were examined with regard to effects of Bt maize plants on this tritrophic system. Sublethal doses of Bt toxin, expressed in the transgenic cultivars, resulted in 79% mortality of late larval instars of *H. armigera*. The cumulative effects of Bt toxin contributed to a high post-larval mortality which resulted in reduced emergence and fecundity of moths. Larval mortality was dependent on a high variability of Bt toxin expression and nutritional value of these plants enabling a higher percentage of larvae to survive on these plants. The eggs from a single moth reared on Bt maize were of poor quality for the egg parasitoid *Trichogramma brassicae* resulting in a low parasitization efficiency of F1 *Trichogramma*.

Keywords: Bt maize*, Helicoverpa armigera, Trichogramma brassicae*, Bt toxin variation, survival, tritrophic interactions

Introduction

Since the commercial release of Bt maize which expresses CryIAb, eight years ago in the United States, a number of studies have been done on the impact and effectiveness of these lines on other Lepidoptera pests (e.g., Archer *et al.*, 2001; Burd *et al.*, 2003; Gould *et al.*, 1997; Horner *et al.*, 2003a, b; Horner & Dively, 2003; Storer *et al*., 2001). Special attention was paid to indegenous heliothines species like *Helicoverpa zea*, a close relative of *H. armigera*, polyphagous on several crops of economic importance with the background of resistance evolution and management. However, these studies revealed only moderate dose effects of Bt maize on *H. zea* possibly increasing Bt-resistance in *H. zea* populations. After the regulatory framework for release and marketing of Bt crops was modified in 2003 by the EU, a commercial release will become possible soon in Europe. *Helicoverpa armigera* is also a serious pest of sweet and field maize in South and Southeast Europe (Dömötör *et al.*, 2002). This region will also be affected by these Bt lines in the same way as it was observed for *Helicoverpa zea*: a few moths will survive as residual population on Bt maize plants (Kiss *et al.*, 2003). Consequently, population survived on these plants will propagate and thus, an increasing possibility of Bt-resistance evolution likely exists. To overcome this problem not only a refuge strategy is favoured and demanded by the EU, but also an additional release of *Trichogramma* species is conceivable. So far, infestations of Lepidoptera pests in maize are controlled by mass releases of *Trichogramma* species in these countries.

As far as we know, there haven't been many studies on Bt effects on *Trichogramma* species due to the fact that the Bt maize was developed to suppress infestations of the European corn borer (*Ostrinia nubilalis*) and consequently, indundative mass releases of *Trichogramma brassicae* (e.g.) become unnecessary in this pest management strategy. Hence, it is not known whether a coexistence of these two different pest management strategies would be workable with respect to resistance management or it would lead to possible adverse effects of Bt toxin on the *Trichogramma* species.

The present study was conducted to investigate the possible effects of Bt maize expressing CryIAb on *Helicoverpa armigera* and the egg parasitoid *Trichogramma brassicae*. We examined different life history traits of *H. armigera* and used the eggs of moth survived on Bt maize for further estimating the parasitism efficiency of 1st generation of *Trichogramma*.

Materials and Methods

Plants

The Bt maize line of Monsanto Company Bt-MON810 expressing CryIAb and its isogenic line were used for the experiments. Plants were grown under greenhouse conditions and fertilized during growing period with 1,5 g N, 0,75 g P_2O_5 and 1,8 g K_2O per plant to initiate development of 1-2 cob per plant.

Insects

Helicoverpa armigera

Eggs of *Helicoverpa armigera* were provided from BBA Darmstadt, Germany and from Bayer CropScience AG, Monheim, Germany. These were unselective, susceptible strains to the Bt toxin CryIAb. The eggs were maintained in a climatic chamber at 27 °C, 70% relative humdity and a photoperiod of 16L: 8D until hatching of larvae. The neonate larvae were transferred to maize plants at growth stage BBCH 65 (silking: silk hairs fully emerged).

Trichogramma brassicae

The parasitoids were obtained from the company BIOCARE, Einbeck, Germany. The *Trichogramma* wasps are reared successfully in the eggs of Angoumois grain moth *Sitotroga cerealella* by BIOCARE (personal communication, W. Beitzen-Heineke, BIOCARE) for augmentative releases to control the European corn borer (*Ostrinia nubilalis*) in maize. The parasitized *Sitotroga* eggs were kept in glass vials (30 ml) at 25 °C, 70% and a photoperiod of 16L: 8D until hatching of adults.

Experimental set up

H. armigera on maize

The neonate larvae of *Helicoverpa armigera* were transferred to silk hairs of the maize plants for feeding on the developing cobs. Due to the high expected mortality of L1 larvae and the effect of Bt toxins the number of larvae infested on Bt maize plants was twice as high as on the isogenic line. Thus, twelve larvae per plant were transferred on the Bt maize and six larvae per plant on the isogenic line. During next three to four weeks the infested plants were daily observed if any larvae, excrements or holes were noticeable and such observations were recorded.

When nearly 1/3 of the larvae were in early prepupae stage or started to search for a pupation place by digging a hole in soil of the pots, this was taken as initial day, termed here as collecting day, to collect all larvae from each plant or from the soil in the pots. All larvae were recorded and kept singly in a ventilated plastic cup (Ø 9 cm) in a climatic chamber at 27°C, 70% relative humidity and a photoperiod of 16L: 8D. Further, all plants were harvested and cobs and leaf material were stored in a cooling room at 2°C to keep food material for the larvae. The larvae were provided with pieces of the respective maize cob on which these were collected. A few leave disks of the maize cobs were additionally placed into the cups to provide the larvae with a refuge for resting. Every second day food was replaced to prevent bacterial and fungal infection. This procedure was carried out until the larvae reached the prepupae stage. The hatched adults reared on the isogenic and Bt maize were separately grouped in cages with maize plants to allow mating and egg laying. These eggs were used for a parasitization experiment to measure the effects of the 1st generation of *Trichogramma* reared Bt and isogenic *Helicoverpa* eggs on its efficiency.

Due to the high mortality of *Helicoverpa* larvae and consequently, the low number of adults who completed their larval development on Bt maize, a second trial of rearing *Helicoverpa* on maize was conducted. In following, these two set ups are mentioned as 1st and 2nd trials of *Helicoverpa* rearing. The 2nd trial was enlarged to

38 plants per plant variety, whereas the 1st trial was set up with 14 plants per variety. The collecting day for 1st trial was on 26th day after infestation and for the 2nd trial on 28th day after infestation.

Parasitism efficiency of *Trichogramma brassicae*

The eggs of females reared on isogenic and Bt maize were collected from the plants and clued on filter paper (ø 9 cm). The filter paper was cut into six pieces containing each 13-18 eggs from females of isogenic line and 3-5 eggs from females of Bt maize line. The pieces were transferred into glass vials (30 ml) and females of *Trichogramma* were exposed at a ratio of 1 parasitoid female to 1 host egg. The eggs were reared further in climatic chamber (27 °C, 70% relative humidity and 16L: 8D) until from the parasitized eggs newly *Trichogramma* hatched. These *Trichogramma* were introduced into boxes with *Helicoverpa* eggs on filter paper, calculating 50 eggs per female for estimating parasitization efficiency. This set up was replicated six times. Boxes were controlled daily to remove possible hatched larvae of *Helicoverpa* preventing cannibalisms to eggs and larvae. On the 5th day the parasitization was estimated by counting the black eggs.

Statistical analysis

The survival rate of *Helicoverpa* larvae per plant was estimated by counting the larvae at the collecting day in relation to over all number of larvae that were infested per plant and variety. Further, it was expected that one larva can develop per ear and plant. The expected proportion of larvae per plant was related to the proportion of larvae that were found at the collecting day presenting the survival to late instars. The survival rates per plant of both varieties were compared by using the Mann-Whitney U test. Further, the Chi-square test was used for analysing differences between the larval stages among the collected larvae from the isogenic and Bt line.

Parasitism efficiency of *Trichogramma* females was calculated as the number of parasitized eggs in relation to the number of total eggs which were calculated per female. Here also the Mann-Whitney U test was used to compare the different ratios. This nonparametric tests was carried out with SYSTAT® Version 10 for Windows®.

Results

Helicoverpa armigera - life history traits

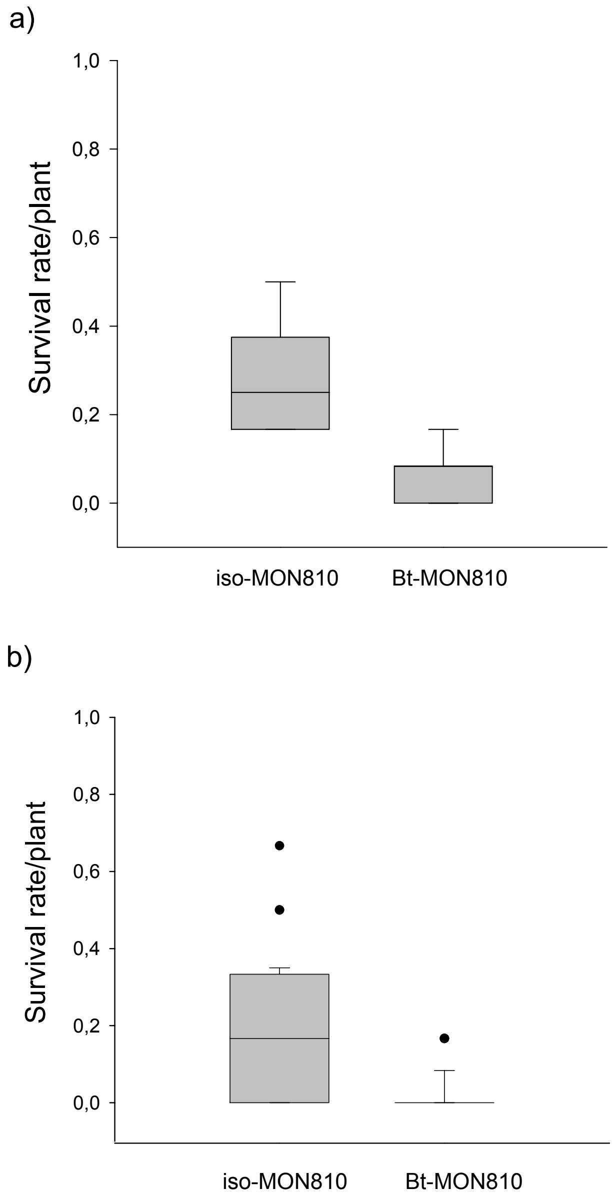

Fig. 1. Survival rate per plant of *Helicoverpa* larvae on Bt-MON810 and isogenic line. a) 1st trial of Helicoverpa rearing (n= 14 plants/line), b) 2nd trial of *Helicoverpa* rearing (n= 38 plants/line).

The survival rate of *Helicoverpa* larvae per plant collected in the third week after infestation was highly significantly different in the first trial (Mann-Whitney U test statistic= 189,00; $P < 0,001$) between larvae from the Bt and isogenic maize line.

This significance was found also in the second experiment (Mann-Whitney U test statistic= 1080,00; $P < 0,001$). Although the number of plants per line was increased to 38 in the 2nd trial, fewer larvae on these Bt plants survived compared to the first trial with 14 plants per line (Fig. 1).

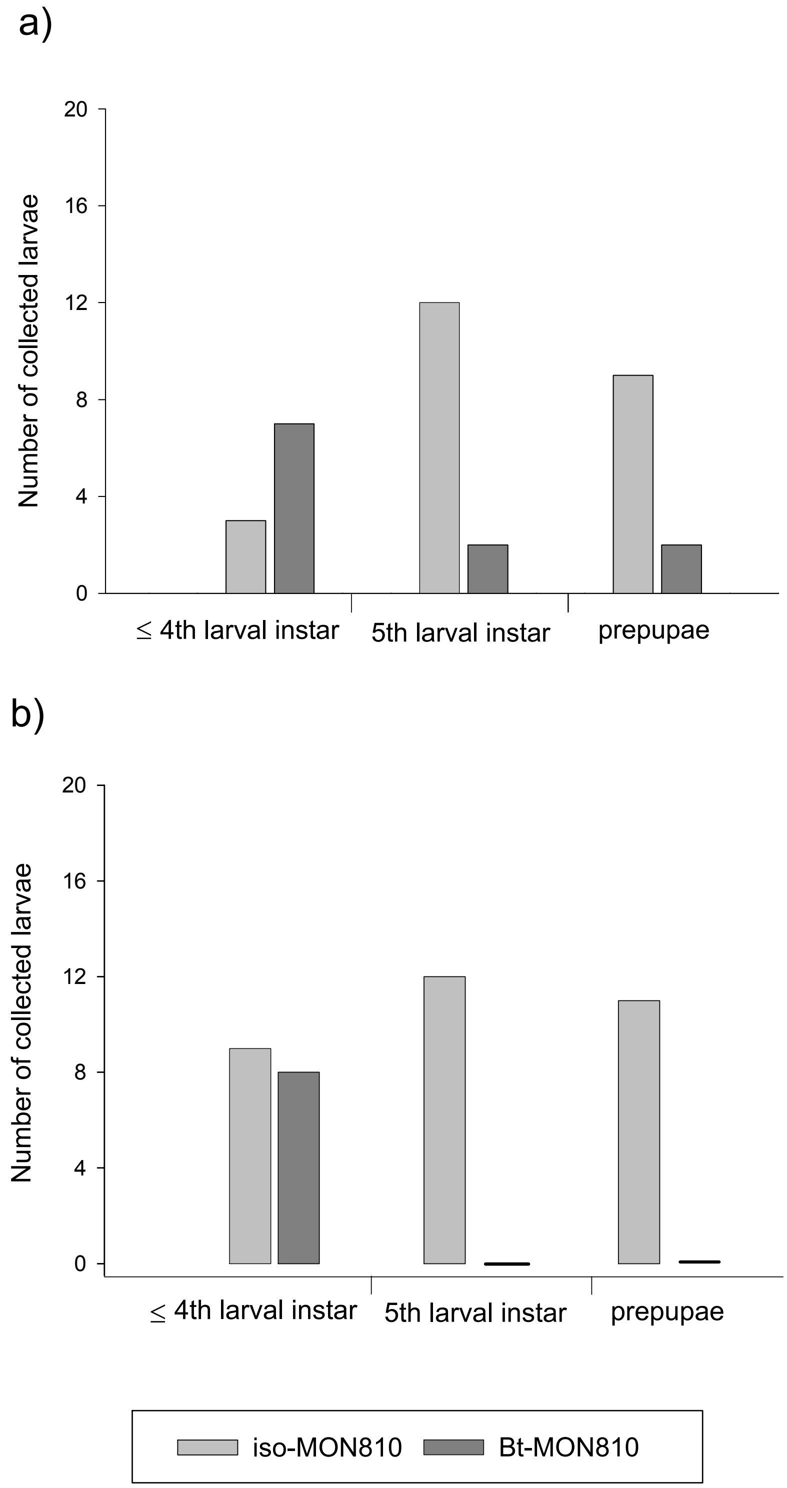

Fig. 2. Larval stages of *Helicoverpa* from Bt and isogenic maize at the collecting day. a) 1st trial of *Helicoverpa* rearing, b) 2nd trial of *Helicoverpa* rearing.

Furthermore, the survival rate of larvae per plant of isogenic line in the 1st trial was slightly higher than the survival rate per plant of the isogenic line in second trial.
Overall, in the 1st trial 1-2 larvae developed on each plant of the isogenic line resulting in a survival to late instars of 171%, whereas on 9 Bt plants 1-2 larvae developed resulting in a survival to late instars of 79%. Moreover, in the 2nd trial
1-2 larvae developed on 22 isogenic plants resulting in a survival to late instars of 84% and 1-2 larvae developed only on 6 out of 38 Bt plants resulting in a survival to late instars of 21%.

The collected larvae from Bt maize and from isogenic line in 1st trial were grouped into different larval stages as follows: only few larvae from Bt maize plants could develop to a stage of 5th larval or prepupae instar (Fig. 2a). Whereas, the larvae from the isogenic line developed into the 5th and prepupae instar in time during the last three weeks. The most larvae on Bt plants were found in earlier larval stages than L4 in both trials, whereas on isogenic plants these larval stages were numerically lower compared to the older larval stages on these plants. In the 2nd trial the larvae on Bt maize developed not to 5th and prepupae larval stage as compared to the larvae on isogenic maize (Fig. 2b). The survived larvae on Bt developed to the 4th or earlier larval stages in the 2nd trial.

The summarized data of larval stages in both trials showed a significant difference between larvae reared on Bt maize cobs and on isogenic maize cobs (Chi-square test= 22,54; df= 2; P < 0,001).

The prepupae weight of *Helicoverpa* larvae on Bt and on isogenic maize plants of the 1st trial were not significantly different (Mann-Whitney U test statistic= 68,50;
P > 0,10). The median prepupae weight of isogenic larvae were 0,354 mg and for the Bt prepupae 0,289 mg. However, the prepupae weight of both lines from the 2nd trial could not been compared due to the mortality of all larvae of the Bt line during this stage.

The postlarval mortality (prepupae – pupae) of isogenic larvae was 41,66% and 63,63% of Bt larvae in the 1st trial. From all 14 pupae (9 male: 5 female) of the isogenic line moths emerged, whereas from 4 pupae (2 male: 2 female) of the Bt maize line only 2 moths emerged.

Thus, 5 females of isogenic line and only 1 female of the Bt line were obtained for producing eggs for experimental set up with *Trichogramma*. Further, the moths reared on Bt maize emerged nearly 3 ½ days later than the adults reared on isogenic line.

Parasitism efficiency of *Trichogramma brassicae*

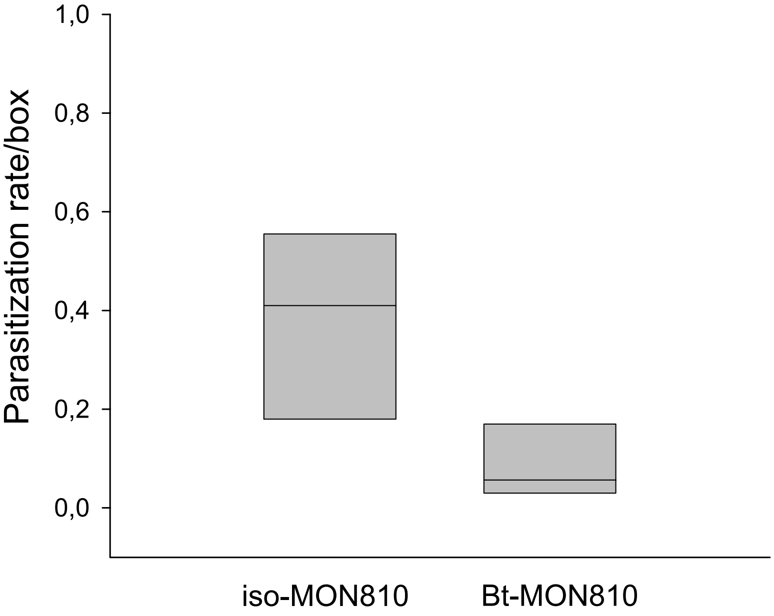

Fig. 3. Parasitism efficiency of the 1st generation of *Trichogramma brassicae* reared on Bt-*Helicoverpa* and isogenic eggs. (Mann-Whitney U test statistic= 35,00; *P* < 0,01).

The collected eggs from females of both varieties were used to rear *Trichogramma brassicae* for further experiments. The newly hatched progeny of this 1st generation of *Trichogramma* on isogenic and Bt *Helicoverpa* eggs were used for this parasitization experiment.

Interestingly, the parasitism efficiency of this first generation of *Trichogramma brassicae* was significantly different (*P* < 0,01). The females reared on Bt *Helicoverpa* eggs had a parasitism efficiency of 5,7% (median), whereas the females reared on isogenic *Helicoverpa* eggs had a parasitism efficiency of 41% (median) (Fig. 3).

Discussion

Helicoverpa on maize

The *Helicoverpa* larvae on isogenic maize in both trials survived with nearly 1-2 larvae per plant, whereas the larvae on the Bt maize plants hardly survived. This was not surprising considering the fact that Bt toxin CryIAb is insecticidal on Lepidoptera pests and expressed in foliage and reproductive structures (AGBIOS, 2001). Horner *et al.* (2003a) estimated mortality of *Helicoverpa zea* larvae to late instars by at least 75% in the field and as reported by Storer *et al.* (2001) mortality of larvae ranged from 60 to 85% in the fields. These results are partly in agreement with our results where 79% of larvae to late instars died on Bt maize in the 2nd trial, which are contradictory to 1st trial where larvae to late instars had a mortality of only 21% on Bt maize. Differences between both trials in survival proportion could be an indication for highly variable Bt toxin contents between and within the plants. Thus, it seems that in the 1st trial half of the Bt plants expressed sublethal or even lower levels of the toxin enabling the larvae to survive. Archer *et al.* (2001) reported that Bt hybrids like Bt-MON810 were not able to control *Helicoverpa zea* on kernels; it was highly variable among hybrids and locations. In addition, Storer *et al.* (2001) concluded that Bt hydrids have only moderate effects on *H. zea* as compared to *Ostrinia nubilalis* due to its less susceptibility to CryIAb. Furthermore, the sublethal effects derive from the lower expression of Bt toxin in the silk tissue and kernels compared to levels in the leaves and stalk, and not all kernels express the same level of endotoxin due to allele segregation resulting in ≈25% of kernels not expressing at all (Horner *et al.*, 2003a). Despite these facts of different levels of Bt toxin in plant tissue and susceptibility to the endotoxin, our results indicate that there would have been an impact of unknown interactions of the plant metabolisms and Bt toxins and/or plant stress on the Bt toxin expression. Thus, the difference can not only be explained by a variation of Bt toxin contents in ears and kernels which is indicated with a range of 0,19-0,39 ug/g (f.w.) protein expressed in kernels and 3,65-4,65 ug/g (f.w.) in whole plant (AGBIOS, 2001) resulting in a moderate dose effect for *Helicoverpa zea* with 60-85% mortality as mentioned above.

Interestingly, in the 1st trial on every second Bt maize plant and in the 2nd trial only on every 6th Bt plant a larvae survived, although the plants were equally treated like using the same soil, fertilizer, temperature and lighting conditions. Further, under greenhouse conditions watering was optimal and consequently, water stress did not occur. However, it was shown that plants react to lighting stress under greenhouse and field conditions due to different light intensity and wavelengths changes (Wu & von Tiedemann, 2004).

Horner *et al.* (2003a) also discussed the possibility of the influence of plant stress during the growing period in maize resulting in a lower efficacy of Bt-MON810 in reducing *Helicoverpa zea* infestations and ear damage.

It was observed that endotoxins of the Bt-MON810 line were highly variable expressed among plants, different sites and years (Hang, 2004). Although it is widely known that the Bt toxin expression in maize is declining over the growing season (AGBIOS, 2001), yet, there haven’t been any evident reports on environmental impacts on the Bt toxin expression as reported for Bt cotton cultivar Ingard® expressing CryIAc (Mahon *et al.*, 2002). Olsen & Daly (2000) concluded in their study that plant-toxin interactions in fruiting cotton reduced the toxicity of the Cry1Ac protein compared to earlier growing stages. Moreover, in squaring Bt cotton the expression of another gene was influenced by the same promoter which also regulates Bt toxin expression (Mahon *et al.*, 2002). Hence, it is possible that Bt toxin expression in maize is also influenced by several unknown factors resulting in interactions of plant metabolism and Bt toxins.

Although, both lines of maize developed almost 2 cobs per plant needed for rearing enough larvae, *Helicoverpa* developed only on 22 out of 38 isogenic plants in the 2nd trial. An explanation could be the high neonate larval mortality which is caused by several movements of larvae searching for suitable food sources (Zalucki *et al.*, 2002). Thus, it seems that some silk hairs were unpalatable and of low nutritive value for neonate larvae caused by earlier maturation of the silk hairs.

Furthermore, the sublethal effects of Bt toxins had consequently an influence on larval development and pupation of the larvae. Only 1/3 of the larvae survived on Bt in the 1st trial reached the 5th larval instar and prepupal stage, while in the 2nd trial none of the larvae reached these stages. The larvae who fed on Bt maize kernels were nearly 2 or more stadia behind larvae who fed on isogenic maize kernels. This was a time developmental delay of approximately 1 to 2 weeks. These findings are similar to the studies of Horner *et al.* (2003a, b) and Storer *et al.* (2001) who also found this proportion of developmental delay of Bt larvae. Further, we observed that 2nd instar larvae feeding on Bt maize were able to survive in this stage for over 2 weeks without growing and consequently, reaching the following instar. Due to the low performance of larvae on Bt maize in the 2nd trial all larvae died during prepupae stage, whereas in the 1st trial nearly 37% of the larvae survived the postlarval stages. The high postlarval mortality was also observed by Storer *et al.* (2001) who indicated a range of 20-40%. However, we had a high postlarval mortality of larvae reared on isogenic maize kernels. The rearing procedures might have contributed to this mortality, because it was reported by Singh (1999) that the nutritive value of artificial maize diets was minimal in relation to artificial soybean diets for *Helicoverpa* larvae. Although the larvae were provided with kernels from the respective maize cob, the lower nutritive value of these and the further maturation process of the maize cobs might have had an influence on the mortality of larvae.

The cumulative effect of the Bt toxins during larval development negatively contributed to the emergence rate of moths and their fecundity which were reduced by at least 50%. Horner *et al.* (2003a) also reported low fecundity of female moths of *Helicoverpa zea* reared on Bt maize which not resulted in any fitness disadvantages in F1 larvae feeding on soybean.

In conclusion, our data revealed an influence of environmental factors to Bt toxin expression in maize. Furthermore, the survival of larvae on Bt maize seem to be very dependent on these variable effects and thus, if larvae survived by chance a prediction of resistance evolution is less likely to become true.

Parasitism efficiency of *Trichogramma brassicae*

It is impossible to conduct parasitization experiments with eggs from *Ostrinia nubilalis* due to nearly 100% mortality of these larvae on Bt maize. Zhang & Zimmermann (2002) studied the effects of Bt corn pollen, phloem sap and honeydew on *T. brassicae.* Neither longevity nor fecundity of *T. brassicae* was influenced by the Bt toxins after several generations.

The result of our experiment revealed a carryover on the parasitism efficiency of *Trichogramma brassicae* which were reared on *Helicoverpa armigera* eggs. The parasitization rate of *Helicoverpa* eggs was significantly reduced in those boxes with

Trichogramma reared on eggs of the Bt moth than with *Trichogramma* reared on eggs of moths reared on isogenic maize. Low fecundity and possibly a related lower egg quality of the single female moth reared on Bt maize seemed to have contributed to this lack of efficiency. Egg quality in terms of suitability for parasitization and development for egg parasitoids is related to size, age and good proteinaceous nutritional value (Bai *et al.*, 1992; Noldus, 1989; Thompson, 1999). Moreover, host recognition and acceptance in *Trichogramma* spp. depends highly on semiochemicals released by plants, hosts, plant-host scales and host eggs (e.g., Boo & Yang, 2000; El-Wakeil, 2003; Gross *et al.*, 1981; Noldus, 1989). Due to these complex plant-host interactions in *Trichogramma* several factors could explain the lack of parasitism efficiency of the 1st *Trichogramma* generation reared on Bt *Helicoverpa* eggs.

One could be that the adults were influenced in their mobility and longevity resulting in low viability and parasitism efficiency due to adverse effects of size and/or insufficient nutritional value of the eggs. In general, females emerged from smaller hosts have low fecundity and lower longevity than females from larger hosts (e.g., Bai *et al.*, 1992; Bigler *et al.*, 1987; Smith, 1996). Another factor could be that the females of low performance were influenced in their olfactory responses and did not accept or were not able to detect the eggs in the proportion than females reared on eggs of moths of the isogenic maize.

Moreover, host acceptance plays a crucial role in indunative mass-releases in biological control programs and is therefore an important quality control parameter for mass-produced *Trichogramma* species (Cerutti & Bigler, 1991; Laing & Bigler, 1991). Even though *T. minutum, T. chilonis* and *T. australicum* are highly effective on heliothines eggs (Aganon & Adhikari, 2004; El-Wakeil, 2003; Jarjees & Merritt, 2002; Nurindah *et al.*, 1999; Smith, 1996), we considered *T. brassicae*, an indigenous species in Central and Southeast Europe, to be also effective on *Helicoverpa armigera* due to its host plant acceptance and large host range (Babendreier *et al.*, 2003; Smith, 1996). Thus, species-specific factors in host acceptance should have not play a role in our experiments, considering the parasitization rate of *Helicoverpa* eggs in boxes with *Trichogramma* reared on eggs of isogenic moths. Hence, the assumption that one female could parasitize up to 50 host eggs was not confirmed in this experiment.

However, which reason might have caused this decline in efficiency, the eggs were obtained from just one single female which might have suffered from negative effects of Bt toxins resulting in a low egg quality. Unfortunately, from the 2nd trial none of the larvae on Bt maize survived to adult emergence and the question remains open whether this observed effect is singularly or will occur to a larger extent. But interestingly, a carryover effect was observed to 1st Trichogramma generation reared on eggs from moths of Bt maize.

In conclusion, following the discussion mentioned above it is known that poor egg quality affects *Trichogramma* development and their viability and therefore, it is not unlikely that a cumulative effect of Bt toxin can affect not only the fecundity, but also the egg quality of female moths susceptible to CryIAb. Another question would be also if an effect on moths will be observed who become resistant to Bt toxins and not survived by chance on the Bt maize plants. Thus, a lot of questions regarding the effects of Bt maize on non-target organisms are still not answered and supporting the demands for a long-term monitoring.

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General discussion

The following discussion contains three questions of the problems which arose during these studies and weren’t reviewed in the chapters.

Understanding parasitoid behaviour

Our previous studies on the behaviour of the parasitoids in the wind tunnel revealed that not only the direct alighting behaviour of the parasitoids on plants should be recorded, but also the no response behaviour which was observed during these tests. This outcome ensues from a logical conclusion that if a parasitoid is able to discriminate between different host plant-herbivore systems it has also the ability not to decide for one of the offered host plant-herbivore systems. The rejection of the both offered systems would lead therefore in no response behaviour. Another possibility for this behaviour could be a highly sensitive response to new environmental influences leading to resting or stress behaviour by either doing nothing or carrying out several non-orientated flights in the wind tunnel.

However, more tests showed that females which had previous experience in the wind tunnel performed better direct flights than females without experience. This is an indication for the associative learning behaviour of female parasitoids discussed by several authors (e. g., Godfray, 1994; Lewis *et al.*, 1990; Mackauer *et al.*, 1996). Consequently, the number of no response representing the range of plasticity of parasitoid behaviour can not be excluded from the analysis as it was done in former behavioural studies (e. g., Du *et al.*, 1996; Guerrieri *et al.*, 1999; Poppy *et al.*, 1997; Schuler *et al.*, 1999).

But this third choice category will have an impact on the data analysis which might lead to unclear results showing not a specific preference for a host plant-herbivore system. Therefore, wind tunnel tests should be linked with other behavioural tests like choice tests in an arena or 4-arm-olfactometer and parasitization tests. Further, parasitoids are not only attracted to volatile cues, but also to visual cues (Michaud & Mackauer, 1995; Vater, 1971). This has to be considered during behavioural tests in the wind tunnel where infested plants show different phenological characters by an increasing duration of the experiments. Thus, the comparability within different dual choice tests can only be guaranteed if these tests including also controls will be completed within a week.

Impacts of Bt transgenes on parasitoid species in general

The studies on the potential effects of Bt transgenes on different herbivorous insect-parasitoid interactions didn't result in any adverse effects for the investigated parasitoid species with the exception to the effects of the Bt maize host plant-herbivore system on the egg parasitoid *Trichogramma brassicae*.

Therefore, our hypothesis that Bt toxin expression will alter the plant metabolism resulting in an effect on parasitoids could not be confirmed for aphid parasitoids. Furthermore, the parasitoids were not attracted to different volatile spectrums of the offered plant-herbivore systems. This could be an indication that the volatile blends did not differ in their quantity and quality to an extent which can favour the parasitoids to prefer a host plant-herbivore system over another. Another explanation could be that Bt toxin expression did not alter the host plant-herbivore cues and were therefore not detectable by the parasitoids. However, further analysis of volatile blends of these varieties may answer this question.

The effects of Bt toxins on herbivorous insect-parasitoid interactions which were observed in studies during previous years (Baur & Boethel, 2003; Blumberg *et al.*, 1997; Chenot & Raffa, 1998; Hafez *et al.*, 1997; Schuler *et al.*, 1999), showed a direct relation of Bt toxins targeted against the herbivorous host and effects on their parasitoids. An exception was the finding by Ashouri *et al.* (2001) which showed an effect of Bt potatoes resistant to Colorado potato beetle, on *Aphidius nigripes* interpretable in terms of bad aphid host quality. Moreover, herbivores resistant to the target Bt toxin enabled also parasitoid larvae to survive (Johnson *et al.*, 1997; Schuler *et al.*, 1999), whereas sublethal doses on target herbivores also had effects on their parasitoids (Chenot & Raffa, 1998). Thus, an effect is likely to occur when hosts are affected at lethal or sublethal doses of the Bt toxins or when host quality is reduced. The studies of Raps *et al.* (2001) and Head *et al.* (2001) revealed no detectable CryIAb in aphids feeding on Bt maize plants due to the fact that Bt toxins are not translocated into pure phloem sap (Raps *et al.*, 2001) and thus, reporting that a harm to parasitoids is unlikely. In conclusion, the results of our studies confirmed these assumptions, the aphid parasitoids were not affected by their hosts feeding on Bt plants. Thus, it seems that the host quality did not alter significantly for the parasitoids. Whereas, the host egg quality of a moth reared on Bt maize plants seemed to have had an impact on the parasitism efficiency of *Trichogramma brassicae* due to the sublethal effects of Bt toxins on this larvae.

Finally, the effect of Bt toxin on parasitoid species depends on the extent to which the Bt plants affect the herbivorous insects. The probability of this effect is greater in parasitoid's target host than the non target.

Bottlenecks, lack of knowledge and outlook

During the studies on effects of Bt maize we observed interactions of plant metabolites of the Bt and non-Bt plants and the herbivorous insects which could not be related to the Bt toxin expression (see chapter 1). It became clear that other resistance mechanisms of plants are still influencing herbivorous insects like toxic secondary compounds such as DIMBOA in maize. As a consequence, the studies had to be adapted to this observed side-effects and if possible to avoid, an interaction of these allelochemicals and Bt toxin expression. It is not known how and to which extent these different resistance mechanisms are influencing one another and contributing to an enhanced or possibly decreased resistance to herbivorous insects. Consequently, the research on potential effects of Bt transgenes is more difficult with this background in sorting out the respective effect influencing the herbivores. Hence, the effects of Bt transgenes in maize should be considered in combination with these observed resistance mechanism of the plants.

Furthermore, effects on herbivorous insect-parasitoid interactions were observed due to different physiological traits of plant varieties during growth period (see chapter 3). This reflects not only further possible influences on a tritrophic system, but also this complex plant metabolism possibly interacting with Bt toxin expression.

The studies on *Helicoverpa armigera* on Bt maize revealed a negative influence of environmental factors on the Bt toxin expression (see chapter 4). This observation give rise to new questions whether the Bt toxin is influenced by such abiotic factors and if yes, when? Moreover, if the Bt toxin expression is affected by abiotic factors and resistance mechanism of plants, the possibility of sorting out Bt effects on the second and third trophic level in the fields under a monitoring plan is becoming more unlikely.

Therefore, effects of Bt transgenes should be investigated under controlled laboratory and semi-field conditions. Even if the trials are carried out in the laboratory and under semi-field conditions, potential effects have to be carefully evaluated with the background of possible interactions with plant metabolites.

Although this present study didn't reveal any adverse effects of these crops on parasitoids the open question remains whether the fecundity and parasitization efficiency in following generations of parasitoids developed on Bt plants will be influenced or not. Therefore, further tests should be conducted within a long-term monitoring program. But our results also showed that a different host preference by aphid parasitoids for hosts feeding on Bt or on alternative host plants is unlikely and therefore, impacts on checking herbivores on these plants can not be expected. Moreover, the effects observed on *Trichogramma brassicae* need to be investigated by conducting further parasitization experiments with host eggs obtained from susceptible and resistant *Helicoverpa* larvae.

In conclusion, the release of Bt crops will become possible soon in Europe and the perception of the public and farmers will decide about an adoption or failure of these crops. Despite this fact the scientific debate about potential impacts on the environment will go on due to several unknown interaction of plant metabolism, Bt toxin expression and possible effects on non-target organisms.

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Summary

Since development and release of genetic modified crops expressing *Bacillus thuringiensis* (Bt) toxins, public concern has raised about the question if Bt plants are safe and have possibly effects on non-target organismen like predators and parasitoids. The aim of this study was to investigate effects of Bt transgenes on herbivorous insect-parasitoid interactions. Three different Bt crop species (Bt maize, Bt canola, Bt eggplants) were tested on their influence on five herbivorous insect-parasitoid interactions. Potential effects of Bt transgenes were estimated on the third trophic level, specifically on parasitoids, using behavioural and performance experiments under laboratory and semi-field conditions. The findings of these studies contribute to the evaluation of potential effects of Bt crops on higher trophic levels and point to bottlenecks and lack of knowledge in this research field.

1) Effects of Bt maize on aphid parasitoid *Aphidius rhopalosiphi*

No evidence was found that neither the foraging efficacy nor the oviposition behaviour of *Aphidius rhopalosiphi* was influenced by a Bt-toxin expression in maize compared to controls plants. The expression of the Bt toxin did not result in any behavioural changes of the parasitoids. Behaviour of the females was strongly related to the volatile cues emanating from uninfested and infested maize plants.

2) Effects of Bt canola on aphid parasitoid *Diaeretiella rapae*

No changes in behavioural responses of the parasitoid *Diaeretiella rapae* to the Bt-toxin expression were observed. Their foraging efficiency was not influenced. Aphid densities did not influence the choice of the parasitoid female with regard to the different canola varieties. Cues emitted by the host plants were of more importance for the searching females than specific cues related to the transgenic event. The expression of the Bt-toxin Cry1Ac did not impact the development of the parasitoids.

3) Effects of Bt eggplants on the parasitoid species *Aphidius ervi* and *Encarsia formosa*

No adverse effects were found for *Aphidius ervi* developing on aphid hosts feeding on Bt eggplants as compared to the isogenic control line. Mortality and longevity of parasitoids from Bt eggplants expressing Cry3B was not different when compared to parasitoids from isogenic eggplants. Different physiological traits during the growing stages of these plant varieties were observed which had an indirect effect on the herbivore-parasitoid-system.

Bt eggplants did not cause any adverse effects on *Encarsia formosa* when parasitizing the whitefly nymphs as compared to isogenic eggplants. The Bt eggplants had not an influence on the development of the *Encarsia*.

4) Effects of Bt maize on *Helicoverpa armigera* and egg parasitoid *Trichogramma brassicae*

Helicoverpa armigera was sublethally affected when feeding on Bt-maize resulting in a mortality of 79% to late instars. Mortality of larvae was dependent to a large portion on variation of Bt toxin expression and nutritional value of these plants. *Helicoverpa* eggs from a moth reared on Bt maize were of poor quality for the egg parasitoid *Trichogramma brassicae* resulting in a low performance of F1 females.

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