Influence of transgenic Bacillus thuringiensis corn-fed prey on prey preference of immature Chrysoperla carnea (Neuroptera: Chrysopidae)

Matthias S. Meier1, Angelika Hilbeck2,*

Swiss Federal Research Station for Agroecology and Agriculture, Zurich Switzerland
1EcoStrat GmbH, Ecological Risk Assessment and Environmental Consulting, Switzerland
2Swiss Federal Institute of Technology, Geobotanical Institute ETH Zurich, Switzerland

Received July 8, 2000 · Accepted August 2, 2000

Abstract

Paired-choice assays in a tritrophic system have been carried out to study the influence of transgenic Bacillus thuringiensis var. kurstaki (Berliner) corn plants on prey preference of the predator Chrysoperla carnea (Stephens). Plants used were a transgenic B. thuringiensis-expressing (Cry1Ab) corn hybrid and the corresponding isogenic untransformed B. thuringiensis-free hybrid. Two different prey species were used in the experiments, Spodoptera littoralis (Lepidoptera: Noctuidae) and Rhopalosiphum padi (Homoptera: Aphidae). Both species were not lethally affected by the Cry1Ab toxin. C. carnea larvae were placed individually in a searching arena together with two groups of prey to choose from. One group had eaten transgenic B. thuringiensis corn (Bt+), the other non-transgenic corn (Bt–). Choice-experiments with various prey and host plant combinations were performed. The number and type of prey consumed by C. carnea, the time C. carnea larvae spent feeding on their chosen prey and the number of prey C. carnea only “probed” upon for one minute or less were recorded. Observations were made for each of the three larval stages of C. carnea. When C. carnea could choose between S. littoralis fed transgenic corn (Bt+) and S. littoralis fed non-transgenic corn (Bt–), they showed a significant preference for S. littoralis fed non-transgenic corn (Bt–) as 3rd instars. Although not statistically significant, a similar trend was observed for the 2nd instar. No preference was observed when C. carnea had the choice between R. padi fed transgenic corn (Bt+) and R. padi fed non-transgenic corn (Bt–). This lack of preference for R. padi fed either transgenic or non-transgenic corn may be due to the absence of the Bt-protein in the phloem. In prey combinations with S. littoralis and R. padi, all three larval stages of C. carnea showed a preference for R. padi regardless whether they had fed on transgenic or non-transgenic corn. These findings are discussed in context with biological control and pest resistance development.

Um den Einfluss von transgenem Bacillus thuringiensis var. kurstaki (Berliner) Mais auf die Beutepräferenz des Räubers Chrysoperla carnea Stephens zu untersuchen, wurden „Paired-Choice“ Assays in einem tritrophischen System durchgeführt. Transgene Maishybriden, die das B. thuringiensis Protein Cry1Ab exprimieren, sowie die entsprechenden isogenen gentechnisch unveränderten Hybriden wurden als Wirts pflanze verwendet. Als Beutetiere dienten Spodoptera littoralis (Lepidoptera: Noctuidae) und Rhopalosiphum padi (Homoptera: Aphidae). Beide Beutetierspezies zeichnen sich dadurch aus, dass sie bei ihnen nach Aufnahme des Cry1Ab Proteins keine letalen Effekte auftraten.

* Corresponding author: Angelika Hilbeck, Swiss Federal Institute of Technology, Geobotanical Institute ETH Zurich, Zürichbergstrasse 38, CH-8044 Zurich, Switzerland; Phone: ++41-1-632 43 22; Fax: ++41-1-632 12 15, E-mail: hilbeck@geobot.umnw.ethz.ch

1439-1791/01/02/01-035 $ 15.00/0
In den Versuchen wurde jeweils eine C. carnea Larve zusammen mit zwei Gruppen von Beutetieren in verschiedenen Kombinationen in einer Sucharena platziert. Die eine Beutetiergruppe hatte zuvor an transgenem Bt M als (Bt+) gefressen, die andere Beutetiergruppe an gentechnisch unverändertem M als (Bt−). Erfasst wurden die Anzahl und die Gruppenzugehörigkeit der von C. carnea gefressenen Beutetiere, die Fresszeit sowie die Anzahl Beutetiere, an denen C. carnea nur „probierte“ (Fresszeiten von einer Minute oder weniger). Die Beobachtungen wurden unabhängig für alle drei Larvalstadien von C. carnea durchgeführt.


Key words: transgenic plant – paired-choice assay – pest management – biological control – pest resistance development

Introduction

Transgenic crops are considered powerful tools in resolving pest management problems and the technology is being adopted very rapidly worldwide. Sales figures of genetically engineered seeds for 1998 are estimated at 1.5 billion U. S. Dollars, and the annual growth rate predicted by market researchers is said to be 30% (Thayer 1999). Major traits of transgenic crops commercially available to date are those conferring resistance to either herbicides or insects or both.

One example is genetically engineered Bacillus thuringiensis corn, which expresses an insecticidal toxin of the gram-positive soil bacteria B. thuringiensis (Bt) in its tissues. The Bt–gene transferred into corn encodes for the 6-endotoxin Cry1Ab specifically targeting lepidopteran pest insects like the European corn borer, Ostrinia nubilalis (Koziel et al. 1993). In susceptible insects the toxin binds to receptors located on the membranes of midgut epithelium cells causing a gradual increase in membrane permeability followed by swelling and lysis of the cells (Schnepf et al. 1998). Through genetic engineering the corn plant acquired a powerful novel defensive compound against certain herbivores. From research on insect-plant interactions (tri- and bitrophic) and food web ecology, we know that chemical host plant defenses can also strongly influence higher trophic level organisms, i.e., natural enemies of these herbivores, in multiple ways.

For example, in tritrophic interaction studies, toxic allelochemicals can pass from plants to natural enemies via their prey (Price et al. 1980, Bernays 1988, van Emden 1995). Herbivore population dynamics can also be affected indirectly via volatiles emitted by plants that are injured by herbivore feeding, thereby attracting parasitoids that kill the herbivores (Bernays & Graham 1988, Vet & Dicke 1992). Another important impact involves changes in the feeding behavior of natural enemies. Traugott & Stamp (1996) showed for example that prey fed an allelochemical-containing diet deterred the predaceous pentatomid bug Podisus maculiventris from killing the caterpillars. Thus, it is conceivable that the high expression of a new potent bioactive compound, such as the Bt toxin Cry1Ab, produced in almost all plant parts from germination to senescence of the plant can exert a significant impact on third trophic level organisms, i.e., natural enemies. With respect to natural regulation of pest populations in crop fields, this can have important consequences for the compatibility of host plant resistance developed by genetic engineering and effectiveness of biocontrol organisms. Because modern agriculture seeks to minimize disruptive effects of natural regulation mechanisms in order to profit from cost-free, naturally-occurring biological pest control, these implications need to be assessed.

In tritrophic laboratory feeding studies, Hilbeck et al. (1998a) documented a significantly increased mor-
tality of larvae of the green lacewing, Chrysoperla carnea (Stephens) when reared on transgenic corn (Bt+) fed prey (Spodoptera littoralis) compared to those reared on prey fed non-transgenic corn (Bt-). Further studies with purified Bt toxins fed either directly to C. carnea via an artificial diet or fed, again, in tri-trophic experiments via the herbivorous prey, S. littoralis, produced similar results (Hilbeck et al. 1998b, Hilbeck et al. 1999). When comparing the Bt concentrations in the various types of diets used in these trials, highest mortality of C. carnea occurred in the tri-trophic experiments using transgenic corn (Bt+). However, these studies did not reveal whether, in addition to the observed lethal effects, also the prey preference of C. carnea larvae was affected. To our knowledge, the feeding behavior of natural enemies has not been investigated to date in a tri-trophic system involving transgenic Bt plants. To date, research has only been conducted on behavior of parasitoids offered susceptible and resistant host larvae raised on transgenic Bt plants (Chilcutt & Tabashnik 1997a and b, Schuler et al. 1999).

A common method for estimating feeding preference in insects is the paired-choice assay (N orldlund & M orrison 1990, Dean & Schuster 1995, Horton 1995). Two food types are offered to a consumer simultaneously. The results are used to test a number of important hypotheses in feeding ecology (i.e., relating to biocontrol capacity) and to understand basic trophic relationships in ecosystems, such as the evolution of host preferences (Ro a 1992, Horton 1995).

The objective of this study was to determine how prey preference of immature C. carnea was influenced by two different prey species (S. littoralis and R. padi) feeding on two different host plant varieties, transgenic corn (Bt+) and the isogenic, non-transgenic corn (Bt-).

**Materials and methods**

**Insect species**

Predaceous larvae of the green lacewing, Chrysoperla carnea, from a permanent laboratory colony were used for the prey preference experiments. C. carnea larvae have been maintained on pea aphids (Acyrthosiphon pisum (Harris) and Ephesia kuehniella (Hübner) eggs since 1988 without any introduction of field collected insects. N on-predaceous adults were kept on a mixture of yeast, honey, and water. Rearing conditions were 22–25 °C (fluctuating), 70% relative humidity, and a photoperiod of 16:8 (L:D) hours.

Two prey species were used: the bird cherry-oat aphid, Rhopalosiphum padi L., and the Egyptian cotton leafworm, Spodoptera littoralis (Boisd). They are both non-target herbivore species that are not or only sublethally affected by Bacillus thuringiensis proteins produced by the HD-1 strain of Bacillus thuringiensis var. kurstaki (Keller et al. 1996, Escriche et al. 1998). S. littoralis larvae are chewing insects ingesting Bt protein when feeding on transgenic corn leaves (Bt+). In contrast, R. padi is a phloem sucking insect, which may not ingest any Bt protein, since it has not yet been conclusively determined whether Bt protein is present in the phloem (Raps et al., unpublished data, presented at the Annual meeting of the Entomological Society of America Atlanta, GA, USA, December 1999).

Approximately 60 winged aphids from the laboratory colony were placed on non-transgenic corn plants (Bt-) in an acrylic glass cage and another 60 winged aphids on transgenic corn plants (Bt+) in another acrylic glass cage. Hence, one colony of R. padi was reared on non-transgenic corn (Bt-) and the other on transgenic corn (Bt+). Corn plants were replaced once per week. Both cages were placed in a growth chamber at a constant temperature of 20 °C, a relative humidity of 70%, and a photoperiod of 16:8 (L:D) hours.

Egg masses of S. littoralis were kindly provided by Novartis, Basle, Switzerland, where they have been maintained as a laboratory colony for several generations. Ostrinia nubilalis, the European corn borer, was obtained from French Agricultural Research, Inc., Lamberton, Minnesota, USA. All egg masses were reared until hatching in the growth chamber with the fluctuating temperature regime (see above).

**Plants**

Two different corn hybrids were used in the experiments. One hybrid (N 4640Bt) was genetically modified containing the truncated, synthetic version of a gene from Bacillus thuringiensis var. kurstaki HD-1 coding for the expression of the insecticidal δ-endotoxin Cry1Ab (Koziel et al. 1993). Transcription of the Bt gene was controlled by the 35S promoter of the cauliflower mosaic virus (CaMV 35S). The mean concentration of Cry1Ab protein in transgenic corn leaves (Bt+) has been reported to range at 4 µg per gram fresh weight during anthesis with peak concentrations of 7 µg per gram fresh weight (Fearing et al. 1997). The other hybrid (N 4640) used was essentially identical to the one described above with the exception that it did not carry the Cry1Ab-gene (Bt-). All plants were cultivated in pairs of the same hybrid in plastic pots in greenhouses. Plant material that was used for the experiments was taken from plants that had reached a height of 40-60 cm (5–7 leaf stage).
Bioassays

To determine if the Bt protein in the transgenic plant material used for the experiments was still biologically active, bioassays were carried out using the susceptible target species Ostrinia nubilalis (European corn borer).

A leaf piece from the transgenic (Bt+) or non-transgenic (Bt–) corn, respectively, was placed into each of ten vials (1.2 cm diameter × 7.5 cm length). Four neonate O. nubilalis larvae were placed into each vial and sealed with perforated plastic lids to allow air circulation (40 larvae per treatment). The vials were then kept in the growth chamber at the fluctuating temperature regime (see above). Bioassays were repeated for each of the nine generations of corn plants used in the choice-experiments. Numbers of dead larvae were recorded after four to five days.

In order to test if the drawing ink used for marking R. padi and S. littoralis had an influence on prey preference of C. carnea, paired-choice assays as described below were carried out but with keeping the variable ‘plant’ (transgenic corn (Bt+) or non-transgenic corn (Bt–)) constant. The two combinations S. littoralis (marked) × S. littoralis (not marked) and R. padi (marked) × R. padi (not marked) were tested for all three larval stages of C. carnea. Each trial was repeated 8 times per plant species.

Paired-choice assays

Tightly closing plastic dishes (5 cm of diameter) were used as searching arenas. Individual C. carnea larvae that had been starved for 20 to 24 hours prior to the observation were placed into the arena together with two groups of prey differing in their food treatment. For R. padi, aphids were taken from the laboratory colonies either reared on transgenic (Bt+) or non-transgenic (Bt–) corn. For S. littoralis, one group of larvae had fed on transgenic corn (Bt+), the other on non-transgenic corn (Bt–) for 12 to 20 hours prior to the experiment. Otherwise, S. littoralis larvae were reared on artificial diet. The various combinations of differently treated groups of prey tested in the experiments are listed in Table 1. When two groups of the same species were used (experiment 1 and 2) one group had to be marked in order to be able to distinguish the two types of prey.

The marking was done with black drawing ink (‘Pelikan’, ‘Drawing Ink A’) just before the beginning of each observation. For S. littoralis a ‘rotring rapidograph’ (Sanford GmbH, Hamburg, Germany) with a 0.18 mm tip was used to put two to three small dots on their back under a dissecting microscope. Since R. padi turned out to be too soft to be marked with this type of pen, a special marking device had to be developed. It consisted of two 3 cm-pieces of fine wire from wire netting used in laboratories. The two wires were glued together on both sides. One end was inserted into a holder, the other was formed into a hook. By dipping the hook into the drawing ink, the space between the wires was filled. When gently stroking over the aphids abdomen some of the ink between the wires attached to their back. This was done under a dissecting microscope.

A possible influence of the drawing ink on prey preference was investigated in separate trials carried out prior to the experiments (see above). However, to compensate for a possible effect, the marking of prey that had fed either on non-transgenic corn (Bt–) or on transgenic corn (Bt+) was alternated.

Paired-choice experiments were carried out separately for all three larval stages of C. carnea. Every second day, C. carnea eggs were taken from the colony and kept under the same rearing conditions as the adults until hatching. Twenty-four hour old, starved C. carnea larvae were used for the experiments with 1st instars. Remaining 1st instar C. carnea larvae not used in the experiments were placed individually in vials (1.2 cm diameter by 7.5 cm length) that were sealed with cotton balls. E. kuehniella eggs were provided as food for these C. carnea larvae. They were kept under the same rearing conditions as above and checked every day for newly molted instars identified by finding the exuvia in the vials. Resulting 2nd and 3rd C. carnea instars were used for further paired-choice experiments on older larvae.

Table 1. Treatment combinations tested in the experiments

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Prey species</th>
<th>Host plant</th>
<th>Prey species</th>
<th>Host plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. littoralis</td>
<td>transgenic corn (Bt+)</td>
<td>S. littoralis</td>
<td>non-transgenic corn (Bt–)</td>
</tr>
<tr>
<td>2</td>
<td>R. padi</td>
<td>transgenic corn (Bt+)</td>
<td>R. padi</td>
<td>non-transgenic corn (Bt–)</td>
</tr>
<tr>
<td>3</td>
<td>S. littoralis</td>
<td>transgenic corn (Bt+)</td>
<td>R. padi</td>
<td>transgenic corn (Bt+)</td>
</tr>
<tr>
<td>4</td>
<td>S. littoralis</td>
<td>non-transgenic corn (Bt–)</td>
<td>R. padi</td>
<td>non-transgenic corn (Bt–)</td>
</tr>
<tr>
<td>5</td>
<td>S. littoralis</td>
<td>transgenic corn (Bt+)</td>
<td>R. padi</td>
<td>transgenic corn (Bt+)</td>
</tr>
<tr>
<td>6</td>
<td>S. littoralis</td>
<td>non-transgenic corn (Bt–)</td>
<td>R. padi</td>
<td>non-transgenic corn (Bt–)</td>
</tr>
</tbody>
</table>

Basic Appl. Ecol. 2, 1 (2001)
Table 2. Numbers of prey used in the experiments for the different larval stages of C. carnea.

<table>
<thead>
<tr>
<th>Larval stage of C. carnea</th>
<th>Number of prey per group</th>
<th>Total number of prey</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>L2</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>L3</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

One day old S. littoralis were used for 1st instar C. carnea, 3 to 4 days old S. littoralis for 2nd and 3rd instar. Accordingly, young aphids were fed to 1st instar C. carnea and larger or adult aphids to 2nd and 3rd instar. Only wingless aphids were used.

Since 1st instars C. carnea feed less and on smaller prey than 3rd instars, numbers and sizes of prey were adjusted to the larval stage (Table 2). Each prey x host plant combination (= 6 treatments, see Table 1) was repeated 20 times (= 20 individual C. carnea larvae) per larval stage (= 3 instars), resulting in 360 observations. Over a four hour observation time, the number and type of prey eaten by each individual C. carnea larva, as well as the feeding time on each prey were recorded continuously. A dissecting microscope was used to determine the type of prey eaten by C. carnea.

Data analysis

For statistical analysis of mortality of O. nubilalis a logistic regression was performed calculating the proportion of individuals that died and accounting for the binomial probability distribution of mortality data. The model used tested for significant replication and treatment effects. Analyses were performed using the GENMOD procedure of the SAS statistical package (SAS Institute 1996, Cary, North Carolina, USA) including a DSCALE and Type 3 statement producing the appropriate F-statistics. In addition, mortality means and standard errors were determined and means were compared by carrying out the MEANS procedure and a LSD test of the SAS statistical package.

Influence on prey preference of C. carnea caused by marking the prey with drawing ink was tested with an analysis analogous to that of prey preference in the paired-choice assay (see below). A Wilcoxon signed rank test was carried out to test for significant differences between the numbers of marked and unmarked prey eaten.

Total number of each of the two prey types eaten per treatment combination and replication was determined and, additionally, the mean number of prey eaten and standard error over all replications was calculated.

Prior to the statistical analysis, prey preference data was divided into prey fed upon for more than one minute and prey fed upon for one minute or less.

Feeding of C. carnea on prey for one minute or less was considered “probing”. No substantial amount of food can be ingested in such brief time. For statistical analysis, two different data sets were created, consisting of either the ‘filtered’ data only (data set without ‘probing’ events) or the ‘unfiltered’ data (data set with ‘probing’ events).

Both data sets were tested for normal distribution using the Shapiro-Wilk test provided by the UNIVARIATE procedure of the SAS statistical package. Since not all data sets were normally distributed, the differences between the total number of prey eaten of each test group were analyzed for significant differences using the Wilcoxon signed rank test provided by the UNIVARIATE procedure. Accordingly the numbers of ‘probed prey’ in each treatment were also tested for significant differences.

For more information regarding the theory of statistical analysis of paired-choice assays see Roa (1992), Manly (1993), and Horton (1995).

Results

Bioassays

The results of the bioassays using the target pest of transgenic corn, Ostrinia nubilalis, confirmed the continuous biological activity of the transgenic corn plants used in the experiments.

During all nine runs, mortality of O. nubilalis fed transgenic corn (Bt+) was significantly higher than when O. nubilalis larvae were fed non-transgenic corn (Bt–) (F = 789.56; df = 1, 170; P = 0.0001). Averaged across all nine replications, mortality of 1st instar O. nubilalis was 98% when feeding on transgenic corn (Bt+) and 18% when feeding on non-transgenic corn (Bt–).

No significant differences in prey preference of C. carnea between marked and unmarked prey was detected, except for one case. Third instar C. carnea larvae fed on R. padi that were raised on transgenic corn (Bt+) exhibited a significant preference for marked over unmarked prey (Wilcoxon: U = -14, P = 0.016).

Paired-choice assay - single prey species on different host plants

S. littoralis on transgenic (Bt+) and non-transgenic corn (Bt–). For the unfiltered data in the prey combinations S. littoralis (Bt–) x S. littoralis (Bt+), no clear statistically significant difference between the number of prey fed non-transgenic corn (Bt–) and the number of prey fed transgenic corn (Bt+) consumed by C. carnea was observed (Figure 1a). However, 2nd and 3rd
instars C. carnea tended to consume more S. littoralis fed non-transgenic corn (Bt–) than S. littoralis fed transgenic corn (Bt+). When analyzing the values of the test statistics for the prey combination S. littoralis (Bt–) x S. littoralis (Bt+), P-values tended to decrease with increasing larval stage of C. carnea but were not significant (1st instar: U = 1.5, P = 0.9501; 2nd instar: U = 29, P = 0.1317; 3rd instar: U = 44, P = 0.1024 [Wilcoxon]).

After excluding the data for prey that C. carnea had only probed upon for one minute or less (=filtered data), the average amount of prey consumed during 4 hours by C. carnea 2nd instar was 5.25 S. littoralis fed non-transgenic corn (also 5.25 in the unfiltered data) and 4.35 S. littoralis fed transgenic corn (4.40 in the unfiltered data), by 3rd instars was 8.55 S. littoralis fed non-transgenic corn (9.20 in the unfiltered data) and 5.90 S. littoralis fed transgenic corn (7.30 in the unfiltered data). This difference between the two groups of prey consumed by 3rd instar C. carnea was statistically significant (Wilcoxon: U = 64, P = 0.0145) indicating a prey preference for S. littoralis fed non-transgenic corn (Figure 1a).

R. padi on transgenic (Bt+) and non-transgenic (Bt–) corn. The analyses of both data sets, unfiltered and filtered, did not reveal any prey preference for either prey type (Figure 1b). Approximately equal numbers of both types of prey were consumed by all 3 instars.

Paired-choice assay – two prey species on different host plants

For the unfiltered data in all prey x host plant combinations, significantly more R. padi than S. littoralis were consumed by 2nd and 3rd instars of C. carnea regardless whether the prey had fed transgenic corn or non-transgenic corn (Figure 2a–d and Table 3). During the 1st larval stage, C. carnea consumed only significantly more R. padi in the prey x host plant combination S. littoralis (Bt–) x S. littoralis (Bt+) (Figure 2a and Table 3).

After filtering the data, the differences between the numbers of S. littoralis and R. padi consumed by C. carnea became even greater, confirming the clear preference of C. carnea for R. padi regardless of the herbivore’s food source (Figure 2a–d and Table 3).

Probing – single prey species on different host plants

S. littoralis on transgenic (Bt+) and non-transgenic (Bt–) corn. For all twenty replications of the experiment, only one 1st and one 2nd instar C. carnea probed on one S. littoralis fed transgenic corn (Bt+). Sixteen out of the twenty 3rd instar C. carnea probed on a total of 28 S. littoralis fed transgenic corn (Bt+) and 15 S. littoralis fed non-transgenic corn (Bt–). However, for none of the three instars the difference between probed prey fed transgenic corn and probed prey fed non-transgenic corn was significant.

R. padi on transgenic (Bt+) and non-transgenic (Bt–) corn. None of the three C. carnea instars showed significant prey probing. However, again, as in the combination S. littoralis (Bt+) x S. littoralis (Bt–), 3rd instar C. carnea showed the highest probing-frequency: Six C. carnea probed on 10 R. padi fed non-transgenic corn (Bt–) and 8 R. padi fed transgenic corn (Bt+).
Influence of transgenic Bacillus thuringiensis corn-fed prey on prey preference of immature Chrysoperla carnea

Figure 2. Stage-specific mean numbers of prey consumed by C. carnea larvae in mixed prey combinations with Spodoptera littoralis x Rhopalosiphum padi: unfiltered and filtered data were analyzed separately (small letters: unfiltered data, capital letters: filtered data). Bars with different letters represent means that are significantly different at $P = 0.05$ (Wilcoxon sign rank test). Error bars indicate SEM.

a) Prey combination Spodoptera littoralis fed non-transgenic corn (S.l. (Bt–))×Rhopalosiphum padi fed transgenic corn (R.p. (Bt+)).

b) Prey combination Spodoptera littoralis fed transgenic corn (S.l. (Bt+))×Rhopalosiphum padi fed non-transgenic corn (R.p. (Bt–)).

c) Prey combination Spodoptera littoralis fed non-transgenic corn (S.l. (Bt–))×Rhopalosiphum padi fed non-transgenic corn (R.p. (Bt–)).

d) Prey combination Spodoptera littoralis fed transgenic corn (S.l. (Bt+))×Rhopalosiphum padi fed transgenic corn (R.p. (Bt+)).

In prey combinations a) and b) unfiltered and filtered bars are the same for L1 since no probing occurred.

Table 3. Test statistics of prey combinations with different prey species for the filtered and the unfiltered data.

<table>
<thead>
<tr>
<th>Prey combination</th>
<th>Larval stage of C. carnea</th>
<th>Unfiltered data Wilcoxon</th>
<th>Filtered data Wilcoxon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>P</td>
<td>U</td>
</tr>
<tr>
<td>S. littoralis (Bt–)× R. padi (Bt+)</td>
<td>L1</td>
<td>-30.50 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>-74 **</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-91 ***</td>
<td></td>
</tr>
<tr>
<td>S. littoralis (Bt+)× R. padi (Bt–)</td>
<td>L1</td>
<td>n.s. n.s.</td>
<td>No probing!</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>-84.5 ***</td>
<td>-72.5 ***</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-73 **</td>
<td>-84.5 **</td>
</tr>
<tr>
<td>S. littoralis (Bt–)× R. padi (Bt–)</td>
<td>L1</td>
<td>n.s. n.s.</td>
<td>n.s. n.s.</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>-80 ***</td>
<td>-80.5 ***</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-80 ***</td>
<td>-103.5 ***</td>
</tr>
<tr>
<td>S. littoralis (Bt+)× R. padi (Bt+)</td>
<td>L1</td>
<td>n.s. n.s.</td>
<td>n.s. n.s.</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>-49 *</td>
<td>-56.5 *</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-76 **</td>
<td>-86 **</td>
</tr>
</tbody>
</table>

n.s. = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.0005$. 

Basic Appl. Ecol. 2, 1 (2001)
Probing - two prey species on different host plants

With increasing larval stage of C. carnea, prey probing increased again, resulting in significant differences between the two prey species during 3rd instars only. Except in the combination S. littoralis (Bt–) × R. padi (Bt+), no significant prey probing occurred for any of the three instars (Table 4). The number of C. carnea larvae showing probing out of the twenty replications in each prey combination varied only slightly for 3rd instars (13–15). In all four prey × host plant combinations, probing occurred always more frequently when C. carnea was feeding on S. littoralis. However, the total number of probed prey and the numbers of probed S. littoralis and R. padi differed considerably between the different prey combinations. The greatest difference was found in the prey combination S. littoralis (Bt+) × R. padi (Bt–) where fifteen 3rd instars C. carnea probed on 31 S. littoralis fed transgenic corn versus 10 R. padi fed non-transgenic corn (Table 4).

Discussion

Single prey species on different host plants

Higher instar C. carnea larvae showed a preference for S. littoralis fed non-transgenic corn (Bt–) when they could choose between S. littoralis fed transgenic corn (Bt+) and S. littoralis fed non-transgenic corn (Bt–). The prey preference for S. littoralis fed non-transgenic corn was highly significant for 3rd instars resulting in approximately 30% reduction in C. carnea prey consumption (filtered data). Lack of significance observed for 1st instars was probably due to the few prey items they consumed during the 4 hours of observation. Second instars showed a trend towards a preference for S. littoralis fed non-transgenic corn (Bt–), but the difference was not significant. These results suggest that C. carnea probed their prey by feeding on it for a brief time (feeding times of 1 minute and less) and left it, if not appropriate, to resume searching for new prey. Usually, probing did not kill the prey during the time of observation. The piercing by C. carnea’s mouthparts caused more or less severe but not necessarily lethal injury of the prey.

Third instar C. carnea exhibited the highest frequency of prey probing. Probing increased after 1 to 2 hours from the beginning of the observation indicating that C. carnea had to eat several prey items first. Either C. carnea had to satiate initial hunger first before allowing itself to choose between prey, or they needed a number of prey items of the various types before developing a preference or acquiring the ability to taste the differently treated prey during the observational period.

Data from the paired-choice assays with S. littoralis alone do not provide an explanation for the mechanism by which C. carnea developed a preference for prey fed non-transgenic corn. Since transgenic corn (Bt+) may exert sublethal effects on S. littoralis (Keller et al. 1996, Escriche et al. 1998) the herbivore may thereby have acquired a suboptimal taste for C. carnea, resulting in the preference for S. littoralis fed

Table 4. Prey probing by Chrysoperla carnea larvae.

<table>
<thead>
<tr>
<th>Prey × host plant combination</th>
<th>C. carnea instar</th>
<th>Numbers of probing</th>
<th>Total number of probed prey for all replications</th>
<th>Wilcoxon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. carnea larvae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. l.</td>
<td>R. p.</td>
</tr>
<tr>
<td>S. littoralis (Bt–) × R. padi (Bt+) 1st</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>13</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>S. littoralis (Bt+) × R. padi (Bt–) 1st</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>5</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>15</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>S. littoralis (Bt–) × R. padi (Bt–) 1st</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>13</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>S. littoralis (Bt+) × R. padi (Bt+) 1st</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>15</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>

n.s. = P > 0.05.
S.l. = Spodoptera littoralis
R.p. = Rhopalosiphum padi

Basic Appl. Ecol. 2, 1 (2001)
Influence of transgenic Bacillus thuringiensis corn-fed prey on prey preference of immature Chrysoperla carnea

Two prey species on different host plants

When C. carnea had the choice between S. littoralis and R. padi as prey species, C. carnea always consumed more R. padi regardless whether R. padi had eaten on transgenic corn or not. It seems that the effect of the transgenic plant on prey preference had become secondary and that R. padi was the more adequate prey for the generalist predator C. carnea, which is supported by many reports in the literature (For review see Bay et al. 1993). When excluding ‘probing’ events, the data revealed an even stronger preference for aphids. Analyzing the ‘probing’ data provides additional evidence for the observed preference behavior. When C. carnea could choose between S. littoralis larvae fed non-transgenic corn (Bt−) and aphids fed transgenic corn (Bt+), no significant probing at all was observed. Conversely, however, when S. littoralis had fed on transgenic corn (Bt+) and aphids on non-transgenic corn (Bt−), C. carnea larvae exhibited significant probing behavior as 3rd instars and yielded the highest number of probed prey item. Although the effect of the transgenic plant on prey preference had become secondary in the mixed prey combinations, it is possible that the effect could become stronger after a longer time of exposure of C. carnea to prey fed transgenic corn.

It is difficult to extrapolate from the observations made in our laboratory trials to the field. In the field usually a community of various herbivore species is present. If aphids are present in a transgenic Bt crop-field, C. carnea would probably feed preferably on these aphids. In the absence of aphids, the composition of the herbivore community present in the system may determine whether C. carnea is adversely affected and will exert reduced biocontrol capacity.

However, our data suggest that C. carnea larvae would prefer to feed on prey containing low levels of or no Bt toxin; or, alternatively, avoid prey containing Bt. Switching to non Bt containing prey where phloem feeders are present, for example, may be a mechanism by which C. carnea could avoid the detrimental effects observed in the no-choice trials conducted by Hilbeck et al. (1998a, 1998b and 1999). Thereby, they would perhaps increase the predation pressure on aphids but reduce their biocontrol capacity for the Bt containing prey. Therefore, these findings may also have implications for pest resistance development. As has been demonstrated in models by Gould et al. (1991), natural enemies can either increase or decrease the rate of adaptation. The models so far considered differing degrees of susceptibility of the pest species reflected in prolonged or shortened developmental times, functional response types of the natural enemies and pest density dependent or independent predation behavior but not selective feeding behavior of predators.

Further investigations are needed on learning behavior of natural enemies, other prey and predatory species as well as experimentation under field conditions. Multitrophic interactions must be carefully considered for the deployment of transgenic plants in sustainable agricultural systems.

Acknowledgements. We gratefully thank Stephan Bosshard and Mario Walburger from the Swiss Federal Research Station for Agroecology and Agriculture for their technical assistance, Novartis Crop Protection Basle, Switzerland for providing the S. littoralis egg masses, Prof. Dr. Peter J. Edwards from the Geobotanical Institute of the Swiss Federal Institute of Technology Zurich, Switzerland for his useful hints regarding data analysis, and Dr. Lisa Pritscher from the Department of Statistics of the Swiss Federal Institute of Technology Zurich, Switzerland for her great support in the statistical analyses.

References


Basic Appl. Ecol. 2, 1 (2001)

Chilcutt CF, Tabashnik BE (1997a) Host-mediated competition between the pathogen Bacillus thuringiensis and the parasitoid Cotesia plutellae of the Diamondback Moth (Lepidoptera: Plutellidae). Environmental Entomology 26: 38–45.

Chilcutt CF, Tabashnik BE (1997b) Independent and combined effects of Bacillus thuringiensis and the parasitoid Cotesia plutellae (Hymenoptera: Braconidae) on susceptible and resistant Diamondback Moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 90: 397–403.


Schne f E, Crickmore N, Van Rie J, Lercules D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998) Bacillus thuringiensis and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62: 775 –806.


