Laboratory studies of the effects of reduced prey choice caused by Bt plants on a predatory insect

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Abstract

Crops transformed to express *Bacillus thuringiensis* (Bt) toxins can cause close to 100% mortality of certain target pest species. This study assessed the effect of target pest reduction on the predatory insect *Chrysoperla carnea* (Stephens) in the presence of alternative prey. Numbers of lacewings recovered from Bt oilseed rape (cultivar Oscar, event O52) did not differ significantly from numbers of lacewings recovered from conventional oilseed rape in cage experiments with the target pest *Plutella xylostella* (Linnaeus) and the non-target pest *Myzus persicae* (Sulzer) when aphid densities were high. However, significantly fewer lacewings were recovered from Bt plants as aphid densities were lowered. Lacewing weights were not affected by plant type.

Keywords: Transgenic plants, biosafety, *Chrysoperla carnea*, *Myzus persicae*, *Plutella xylostella*, *Brassica napus*

Introduction

Crops transformed to express toxins of *Bacillus thuringiensis* (Bt) can provide very high levels of control of certain target pest species (Carozzi & Koziel, 1997; Obrycki *et al.*, 2001). Natural enemies that specialize in attacking such target pests may experience local prey depletion in such crops but more generalist natural enemies may be less affected if sufficient alternative prey is present (Obrycki *et al.*, 2001). The quantification of individual factors affecting interactions between plants, herbivores and predatory insects is made difficult in the field by the complexity of food webs, the variation introduced by changes in environmental conditions and the small size of the study organisms. Laboratory studies carried out in large cages can make a valuable contribution to investigating these effects as they allow a reduction in complexity and standardization of environmental conditions (Schuler *et al.*, 2001).

The objectives of the present study were to investigate effects of target pest removal by a transgenic plant expressing a Bt toxin on a generalist predator in the presence of alternative prey, and to develop methodology for the risk assessment of transgenic plants. Laboratory experiments involved the predatory lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and transgenic oilseed rape, *Brassica napus* (Linnaeus) (Brassicaceae), expressing the Bt toxin Cry1Ac, which is highly toxic to larvae of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) but has no direct effect on aphids such as the peach–potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Schuler *et al.*, 2001, 2003).

Materials and methods

Experimental plants

The Bt oilseed rape (*B. napus* cv. Oscar) transformation event O52 used for this study expressed a truncated

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synthetic Bt cry1Ac gene under the control of the 35S promoter (Stewart Jr. *et al.*, 1996). Untransformed plants of the parent cultivar Oscar were used as controls. Plants were grown in 1 litre pots in a glasshouse set at 20°C and a minimum day length of 14 h. Bt toxin concentrations in leaves were quantified with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Elkhart, USA) as described previously (Schuler *et al.*, 2001). Plants were checked daily and any flower buds removed, as required by statutory biosafety rules.

Insects

A culture of *P. xylostella* (Philippine strain) that had been kept at Rothamsted for over ten years without exposure to Bt or other insecticides was maintained on oilseed rape plants at 22 ± 3°C and a light:dark cycle of 16:8 h. A parthenogenetic culture of *M. persicae* (clone 1076A) was maintained on oilseed rape at 18 ± 2°C and a light:dark cycle of 16:8 h. First instar *C. carnea* larvae were obtained from Koppert Biological Systems UK Ltd and used for experiments on the day of arrival. During transport, *C. carnea* larvae had been supplied with eggs of the moths *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) as a food source.

Experiments with two prey species in field simulator cages

Four experiments were conducted, in which the availability of aphid prey to *C. carnea* larvae was gradually reduced. In the first two experiments plants were each infested with 30 apterous *M. persicae* adults. Experiments 3 and 4 were started with 15 and 8 apterous *M. persicae* adults per plant, respectively. All other parameters remained constant. Field simulator cages measuring $1.7 \text{ m} \times 1.2 \text{ m} \times 1.0 \text{ m}$ were used as described previously (Schuler *et al.*, 2001). Four cages were used in each experiment and three transgenic plus three control plants were placed inside each cage. The plants in each cage formed two rows of three plants, transgenic plants always alternating with control plants. Each plant represented an independent replicate. Plants were 5–6 weeks old at the start of an experiment. Experiments were conducted using a light:dark regime of 16:8 h, a room temperature of 21 ± 1°C (resulting in 26 ± 4°C inside the cages) and ambient relative humidity conditions.

Aphids were allowed to reproduce for 11 days before 100 two- to three-day-old *P. xylostella* eggs were placed on each plant. Two days later 20 first instar lacewing larvae were placed on each plant. Plants did not touch each other and were placed in trays filled with water to prevent insects from moving between plants. The experiments were terminated nine days after release of lacewing larvae (just before *P. xylostella* adults emerged from their pupae) and numbers of insects per plant assessed. Lacewings recovered were flash frozen and then dried at 60°C for 48 h before dry weights were taken.

In each experiment, *P. xylostella* egg viability was assessed by monitoring the hatch rate of 300 to 400 surplus eggs of the same egg batch as used in the experiment. Hatch rates in the four experiments ranged between 89% and 98%.

A preliminary experiment had shown that lacewing recovery rates were very low if no shelters for lacewings to hide and rest during the day were provided. To ensure a reasonable recovery rate of lacewing larvae in the present study, two corrugated cardboard shelters (each consisting of a piece measuring $8 \text{ cm} \times 13 \text{ cm}$ rolled up around a wooden stick) were inserted in each pot. In addition, two layers of filter paper (24 cm diameter, with a hole cut in the centre for the plant stem) were placed around the base of each plant.

At the end of experiment 3, subsamples of aphids were collected from each plant, flash frozen and tested for the presence of Cry1Ac by ELISA as described above.

Statistical analysis

The numbers of *C. carnea* recovered from each of the Bt and non-transgenic plants (out of the initial 20 lacewings per plant) were compared using a generalized linear model with binomial error and logit link (logistic regression). When significant overdispersion was indicated, plant type differences were tested by comparing the ratio of the treatment and residual mean deviances against an F-distribution. *Chrysoperla carnea* mean dry weights were compared between plant types using analysis of variance with variability partitioned into that due to cages (blocks), plants (plots or replicates) within cages (treatment stratum in which plant type effects are estimated), and individual larvae (pseudo-replicates) on plants. ELISA test results for aphids (ng toxin per mg total protein) were analysed similarly. Correlation coefficients were used to describe relationships between numbers of *C. carnea* and *P. xylostella* (total numbers and pupae only) for both plant types together and for non-transgenic plants alone.

Results

No *P. xylostella* larvae or pupae were recovered from Bt plants. Some small feeding holes of first instar larvae were visible in leaves of Bt plants but no larger feeding holes were observed, indicating that no *P. xylostella* larvae survived past the first instar on these plants. Mean percentages of *P. xylostella* recovered (either as larvae or pupae) from non-transgenic plants were 39.5%, 34.1%, 43.8% and 29.9% for experiments 1–4, respectively (out of 100 *P. xylostella* eggs placed on each plant at the beginning of the experiment). Some dead *P. xylostella* larvae were observed in the water trays indicating that a proportion of larvae had tried to emigrate from the plant or had fallen off following disturbance.

Aphid densities in each of the four experiments increased to several thousand aphids per plant, but were too high and aphid distribution across different leaves of the plants too patchy for an accurate assessment of final aphid numbers. ELISA test results of aphids feeding on Bt plants did not differ significantly from those of aphids feeding on non-transformed plants ($F_{1,19} = 1.04$, $P = 0.321$), suggesting that aphids did not ingest Cry1Ac when feeding on Bt oilseed rape. The Bt plants from which the aphids were collected contained between 0.007% and 0.021% Bt toxin of total leaf protein.

Most *C. carnea* recovered were found sheltering under the filter paper collar, dry leaves, the rim of the plant pot or within the cardboard shelters. Some dead *C. carnea* larvae were observed in the water trays indicating that a proportion of lacewing larvae had attempted to leave the plants or had fallen off. The proportion of *C. carnea* recovered from plants varied with experiment and plant type (fig. 1). No effect of
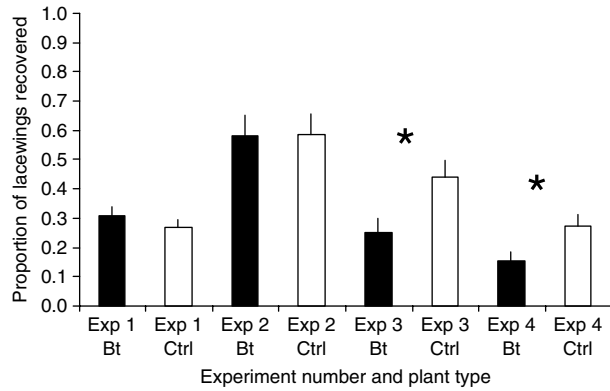
Fig. 1. Proportions of *Chrysoperla carnea* recovered at the end of four experiments. Twenty *C. carnea* were placed on each plant at the beginning of each experiment. Initial densities of *Myzus persicae* were 30 aphids per plant in experiments 1 and 2, 15 aphids per plant in experiment 3 and 8 aphids per plant in experiment 4. Each plant also received 100 *Plutella xylostella* eggs. Error bars represent standard errors based on the binomial distribution. An asterisk indicates a significant difference between Bt and non-transgenic control plants at $P < 0.05$.

plant type on the numbers of lacewings recovered was observed in experiment 1 ($\chi^2_1 = 1.03$, $P = 0.311$; fig. 1). More lacewings were recovered in experiment 2 but again there was no significant difference between plant types ($F_{1,19} = 0.00$, $P = 0.967$; fig. 1). When the data sets of experiments 1 and 2 were combined for statistical analysis, there was no evidence of a significant effect of plant type on proportion of *C. carnea* recovered ($F_{1,39} = 0.14$, $P = 0.712$). However, the proportion of *C. carnea* recovered in experiment 3, in which initial aphid densities were halved, was significantly lower on Bt plants compared to non-transgenic plants ($F_{1,19} = 5.78$, $P = 0.027$; fig. 1). In experiment 4, in which initial aphid densities had been reduced even further, the proportion of lacewings recovered was again significantly lower on Bt plants compared to non-transgenic plants ($F_{1,19} = 5.14$, $P = 0.035$; fig. 1).

No significant effect of plant type on final weights of *C. carnea* was observed in any of the four experiments (table 1). The majority of *C. carnea* were in their last larval instar (81–97%) when the experiments were terminated.

Some (0–19%) were still in the second larval instar and some (0–14%) larvae had already spun a cocoon.

Numbers of *C. carnea* recovered were negatively correlated with total numbers of *P. xylostella* in experiment 1 but not experiment 2 or the combined analysis table 2. There was a positive correlation between these two variables in experiments 3 and 4 (table 2). When the data from Bt plants were excluded, numbers of *C. carnea* recovered were negatively correlated with total numbers of *P. xylostella* recovered in experiments 1 and 2 but there was no statistically significant correlation between these two variables in experiments 3 and 4 (table 2).

Chrysoperla carnea numbers were negatively correlated with numbers of *P. xylostella* pupae in the combined analysis of the first two experiments (table 2). However, the correlation between these two variables was significantly positive in experiment 4 (table 2). Numbers of *C. carnea* per non-transgenic plant were negatively correlated with numbers of *P. xylostella* pupae in the combined analysis of the first two experiments but there was no statistically significant correlation between these two variables on non-transgenic plants in experiments 3 and 4 (table 2).

Discussion

Recovery rates and weight gain of *C. carnea* did not differ between Bt plants and non-transgenic plants in the two experiments that had the highest density of *M. persicae* (experiments 1 and 2). However, significantly fewer *C. carnea* were recovered from Bt plants compared to non-transgenic plants in experiments with lower aphid densities (experiments 3 and 4). For most of the duration of the experiments, the only prey available to lacewings on the Bt plants were aphids and other lacewings since none of the *P. xylostella* larvae appeared to have survived on Bt plants past their first instar. The high mortality of *P. xylostella* on Bt plants was as expected based on earlier studies with the same Bt plant line (Schuler *et al.*, 2003). In contrast, on non-transgenic plants, lacewings had a choice between feeding on aphids, lacewings or moth larvae and pupae. As *C. carnea* larvae are cannibalistic, increased movement due to a more limited supply of aphid colonies may have resulted in higher lacewing mortality due to cannibalism. The differences in *C. carnea* recovery rates in the four experiments suggest that the 'removal' of *P. xylostella* by the Bt plant may only affect lacewings at low but not at high aphid populations.

Table 1. Dry weights of *Chrysoperla carnea* larvae recovered from Bt and non-transgenic control plants. The four experiments investigated the indirect effects of Bt plants on *C. carnea* at different densities of *Myzus persicae*.

Experiment	Plant type	Initial aphid density per plant	Mean (mg)	sem	n	F (df)	P
1	Bt	30	1.86	0.180	74	2.82 (1, 19)	0.109
	Control		2.26	0.194	64		
2	Bt	30	2.95	0.151	140	0.85 (1, 18)	0.369
	Control		2.74	0.151	140		
1 and 2 combined	Bt	30	2.58	0.118	214	0.01 (1, 38)	0.921
	Control		2.58	0.121	204		
3	Bt	15	2.70	0.259	60	1.73 (1, 18)	0.205
	Control		3.16	0.197	103		
4	Bt	8	2.87	0.224	37	0.03 (1, 18)	0.868
	Control		2.95	0.169	65		

Table 2. Correlations between numbers of *Chrysoperla carnea* and numbers of *Plutella xylostella* recovered per plant in four experiments differing in the initial density of *Myzus persicae*.

Plants	Experiment	Initial aphid density per plant	*n*	Total *P. xylostella* recovered		*P. xylostella* pupae recovered	
				r	*P*	r	*P*
Bt and control plants	1	30	24	−0.429	*	−0.455	*
	2	30	24	−0.207	ns	−0.085	ns
	1 and 2 combined	30	48	−0.223	ns	−0.307	*
	3	15	24	0.412	*	0.378	ns
	4	8	24	0.442	*	0.430	*
Control plants only	1	30	12	−0.582	*	−0.59	*
	2	30	12	−0.688	*	−0.218	ns
	1 and 2 combined	30	24	−0.578	**	−0.586	**
	3	15	12	−0.103	ns	−0.195	ns
	4	8	12	0.182	ns	0.317	ns

*$P < 0.05$; **$P < 0.01$; ns, $P > 0.05$.

Previous studies have shown that larval mortality of *C. carnea* increased when larvae were fed the lepidopteran herbivore *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) reared on Bt maize expressing Cry1Ab but not when they were fed the aphid *Rhopalosiphum padi* Linnaeus (Hemiptera: Aphididae) or the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) reared on the same Bt maize (Dutton *et al*., 2002; Hilbeck *et al*., 1998). In the present study, the lack of statistically significant differences between lacewing recovery rate or weight gain on Bt plants compared to non-transgenic plants in experiments 1 and 2 indicates that the lacewings were not directly affected by Cry1Ac expressed in the Bt plants used in the current study. In fact, it is probably unlikely that lacewings on Bt oilseed rape plants were exposed to Cry1Ac during the experiments. Although larvae of *P. xylostella* have chewing mouth parts and ingest leaf cells containing Cry1Ac, the *P. xylostella* larvae feeding on Bt plants died soon after hatching. It appears unlikely that small moribund neonate *P. xylostella* larvae would have represented a significant proportion of a lacewing's prey. Also, Bt-susceptible *P. xylostella* larvae consume only very limited amounts of Bt oilseed rape leaf tissue before death (Schuler *et al*., 2003). Aphids do feed and develop normally on Bt oilseed rape (Schuler *et al*., 2001) but the ELISA test results suggest that *M. persicae* did not ingest Bt toxin when feeding on Bt oilseed rape plants. Similar results have been reported for aphids of the genus *Rhopalosiphum* on Bt maize (Head *et al*., 2001; Raps *et al*., 2001). Aphids have sucking mouthparts and feed predominantly on phloem sap. Bt toxins may therefore not be present in the phloem of Bt maize and Bt oilseed rape.

When attacked by natural enemies, *P. xylostella* larvae often exhibit a violent thrashing behaviour (Schuler, unpublished observations). They also frequently try to escape natural enemy attack by spinning a silk thread and letting themselves drop off the plant (Potting *et al*., 1999). A previous study assessing the preference of *C. carnea* for aphid and moth larvae in choice tests with *R. padi* and *S. littoralis* reported that lacewing larvae preferred to feed on *R. padi* (Meier & Hilbeck, 2001). Lacewings in the present study may therefore have preferentially attacked aphids rather than *P. xylostella* larvae.

Numbers of lacewings and total numbers of *P. xylostella* were significantly negatively correlated on non-transgenic plants in the first two experiments, suggesting a significant impact of lacewings on the *P. xylostella* population, either through predation or through disturbance. However, why the correlation was only significant at the higher aphid densities and not at the lower aphid densities remains unclear.

Pupae of *P. xylostella* became available as prey on non-transgenic plants during the last few days of each experiment as *P. xylostella* larvae reached maturity. Lacewings were repeatedly found feeding on *P. xylostella* pupae. Since *P. xylostella* pupae are similar in size to fully grown lacewing larvae and are unable to escape or physically defend themselves, they appear to be low risk–high return prey for *C. carnea*.

Large numbers of aphids were not only observed in experiments 1 and 2 but also at the termination of experiments 3 and 4, indicating that even in the latter experiments the lacewings were not able to detect or reach and consume all aphid colonies on a plant. The reduced recovery rates of lacewings on Bt plants in experiments 3 and 4 may have been partly due to prey being more dispersed, requiring more lacewing movement and increasing the chances of the predators encountering each other.

The differences in lacewing recovery rates observed in the present study could be due to either lower lacewing survival or changes in predator behaviour that increased lacewing dispersal. Waxy leaf surfaces of *Brassica* species are more difficult for lacewings to adhere to and walk on than glossy leaf surfaces (Eigenbrode *et al*., 1999). Lacewings have been reported to avoid walking on lower leaf surfaces of plants with waxy leaves (Eigenbrode *et al*., 1999). One explanation for differences in lacewing recovery rates observed in the present study may be that as aphid numbers were reduced lacewings did not find enough prey on the upper leaf surfaces and more lacewings fell off the plants while trying to reach prey on the lower leaf surfaces; an effect that may well have been more pronounced on Bt plants than control plants due to the absence of *P. xylostella* larvae on Bt plants.

The present study demonstrates that prey reduction through Bt plants may affect predatory insects negatively

even at relatively high densities of alternative prey. However, the results also suggest the presence of more complex density-dependent behavioural herbivore–herbivore and herbivore–predator interactions.

Acknowledgements

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