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# Tritrophic choice experiments with Bt plants, the diamondback moth (*Plutella xylostella*) and the parasitoid *Cotesia plutellae*

Tanja H. Schuler[1,*], Roel P.J. Potting[3], Ian Denholm[1], Suzanne J. Clark[2], Alison J. Clark[1], C. Neal Stewart[4] & Guy M. Poppy[5]

[1]*Division of Plant and Invertebrate Ecology;* [2]*Biomathematics Unit, IACR-Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK*

[3]*Laboratory of Entomology, Wageningen University, 6700 EH Wageningen, The Netherlands*

[4]*Department of Plant Sciences and Landscape Systems, The University of Tennessee, Knoxville, TN 37901, USA*

[5]*Division of Biodiversity and Ecology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK*

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## Abstract

Parasitoids are important natural enemies of many pest species and are used extensively in biological and integrated control programmes. Crop plants transformed to express toxin genes derived from *Bacillus thuringiensis* (Bt) provide high levels of resistance to certain pest species, which is likely to have consequent effects on parasitoids specialising on such pests. A better understanding of the interaction between transgenic plants, pests and parasitoids is important to limit disruption of biological control and to provide background knowledge essential for implementing measures for the conservation of parasitoid populations. It is also essential for investigations into the potential role of parasitoids in delaying the build-up of Bt-resistant pest populations. The diamondback moth (*Plutella xylostella*), a major pest of brassica crops, is normally highly susceptible to a range of Bt toxins. However, extensive use of microbial Bt sprays has led to the selection of resistance to Bt toxins in *P. xylostella*. *Cotesia plutellae* is an important endoparasitoid of *P. xylostella* larvae. Although unable to survive in Bt-susceptible *P. xylostella* larvae on highly resistant Bt oilseed rape plants due to premature host mortality, *C. plutellae* is able to complete its larval development in Bt-resistant *P. xylostella* larvae. Experiments of parasitoid flight and foraging behaviour presented in this paper showed that adult *C. plutellae* females do not distinguish between Bt and wildtype oilseed rape plants, and are more attracted to Bt plants damaged by Bt-resistant hosts than by susceptible hosts. This stronger attraction to Bt plants damaged by resistant hosts was due to more extensive feeding damage. Population scale experiments with mixtures of Bt and wildtype plants demonstrated that the parasitoid is as effective in controlling Bt-resistant *P. xylostella* larvae on Bt plants as on wildtype plants. In these experiments equal or higher numbers of parasitoid adults emerged per transgenic as per wildtype plant. The implications for integrated pest management and the evolution of resistance to Bt in *P. xylostella* are discussed.

## Introduction

Studies of the effect of insect-resistant transgenic plants on non-target insects have so far predominantly concentrated on small-scale laboratory experiments or on large-scale field trials. Small-scale laboratory studies are often designed to represent worst-case scenarios since they primarily aim to determine the inherent susceptibility of a non-target insect to the transgene product (e.g., Hilbeck et al., 1998; Birch et al., 1999; Losey et al., 1999; Zwahlen et al., 2000).

*Author for correspondence
*E-mail:* Tanja.Schuler@bbsrc.ac.uk

However, this type of experiment only represents a first step towards assessing the side effects of transgenic plants on non-target organisms. Results of such studies usually cannot be used directly to forecast effects on non-target populations in the field, where many other factors, such as insect behaviour, variation in the level of exposure, competition and other mortality factors, come into play (Poppy, 2000; Schuler et al., 2000). Large-scale field studies represent the most realistic scenario but patchy and fluctuating insect populations combined with limited replication make it difficult to detect small effects on non-target organisms at this scale.

There is therefore a need for an intermediate scale of experimentation, which incorporates more realism than small-scale studies but at the same time allows for appropriate replication and control over insect populations. Some recent studies have already demonstrated the potential of this approach. Behavioural choice tests with maize expressing the Cry1Ab toxin of *Bacillus thuringiensis* (Bt) and larvae of the predatory lacewing *Chrysoperla carnea* demonstrated that the predator preferentially feeds on aphids rather than on lepidopteran larvae (the targets of the Bt toxin) (Meier & Hilbeck, 2001). This preference will reduce the exposure of *C. carnea* to Cry1Ab toxin since aphids do not ingest the toxin when feeding on Bt maize (Raps et al., 2001). Population-scale laboratory experiments with the aphid *Myzus persicae* and the aphid parasitoid *Diaeretiella rapae* showed that *D. rapae* was as effective in controlling this non-target pest on Bt and proteinase inhibitor oilseed rape plants as on untransformed plants (Schuler et al., 2001). Glasshouse trials with potatoes expressing snowdrop lectin (GNA), the moth *Lacanobia oleracea* and the parasitoid *Eulophus pennicornis* indicated that the partially resistant GNA potatoes can augment biocontrol of *L. oleracea* by *E. pennicornis* (Bell et al., 2001).

Arthropod natural enemies differ widely in their ecology and specialisation (Jervis & Kidd, 1996), and the indirect effects of transgenic plants on their populations will differ accordingly. Some are specialists that predominantly develop on one host species (e.g., many larval endoparasitoids) while other natural enemies attack a wider range of hosts (e.g., many egg parasitoids) or prey (most predatory arthropods). Populations of generalist predators are least likely to be negatively affected by the absence of the target pest in a Bt plant crop (as they will be able to find other prey) while natural enemies specific to larvae of the target pest present the other end of the spectrum (Obrycki et al., 2001).

A range of brassica crops have been engineered to express Bt toxin genes to provide resistance to the diamondback moth (*Plutella xylostella*, Lepidoptera, Plutellidae) and other lepidopteran pests (Li et al., 1995; Metz et al., 1995a,b; Stewart et al., 1996; Jin et al., 1999, 2000; Zhao et al., 2000; Kuvshinov et al., 2001). *P. xylostella* is a major pest of brassicas and highly susceptible to a number of Bt toxins. However, this pest has developed resistance to a wide range of insecticides including, in some areas, to microbial Bt sprays (Talekar & Shelton, 1993; Tabashnik et al., 1997).

One of the main natural enemies of *P. xylostella* is *Cotesia plutellae* (Hymenoptera, Braconidae) (Talekar & Shelton, 1993), a solitary larval endoparasitoid that is generally reported to be host-specific to *P. xylostella* (Verkerk & Wright, 1996), although there are a few records on other lepidopterous hosts (e.g., Kaneko, 1993; Lipa et al., 1993). Parasitic insects such as *C. plutellae* complete their larval development in a single host insect, eventually leading to the host's death (Godfray, 1994). Their fate is therefore strongly linked with the fate of the host, at an individual as well as a population level, and both direct and indirect effects of transgenic plants on parasitoids have to be considered.

Bt oilseed rape caused 100% mortality of susceptible *P. xylostella* larvae and small-scale no-choice experiments have shown that adult *C. plutellae* females oviposited into such susceptible hosts but premature host mortality on Bt plants prevented the parasitoid larvae from successfully completing their development. In contrast, *C. plutellae* developed normally in larvae of a Bt-resistant *P. xylostella* strain (Schuler et al., 1999; Schuler et al., unpublished data).

The present study introduced choice into experiments investigating the interactions between Bt plants and *C. plutellae*, thereby incorporating behaviour of the non-target organism. Behaviour plays a major role in the success of parasitoids. For example, a female not only faces the formidable task of locating a 'hidden host' in a complex environment but also has to decide whether a host is suitable for the development of her offspring (Turlings et al., 1990; Vet & Dicke, 1992). Parasitoids use chemical stimuli from their herbivorous hosts or the host's food plant to locate hosts. Although host-derived stimuli are reliable indicators of host presence, they are, with the exception of host pheromones, difficult to detect at long distance and are mostly used as contact stimuli. In contrast,

plant-derived stimuli are released in large amounts, are generally more easily detectable at a long distance and can be plant species and genotype-specific (Vet et al., 1991; Vet & Dicke, 1992). Many parasitoids, including *C. plutellae*, discriminate in-flight between uninfested and host-infested plants (Potting et al., 1999). Transgenic plants may differ in their volatile profile from their untransformed counterpart, which may lead to an interruption of the host location process by parasitoids, or alternatively, an ability to differentiate between transgenic and wildtype plants may allow parasitoid females to avoid unsuitable hosts.

Thus, the present study investigated if (a) parasitoid adult females differentiate between Bt plants and untransformed wildtype plants when seeking host larvae, (b) they are more attracted to plants damaged by Bt-susceptible or Bt-resistant *P. xylostella* larvae, and (c) the plant type affects the efficacy of *C. plutellae* to control *P. xylostella* on a mixture of Bt and wildtype plants. Some of the results of the wind tunnel study detailed below have been summarised elsewhere (Schuler et al., 1999) but the present paper represents the first detailed account of these experiments.

## Materials and methods

### *Experimental plants*

The Bt oilseed rape (*B. napus*) line Oscar O52 used for this study expressed a truncated synthetic Bt *cry1Ac* gene, patterned after the Bt ssp. *kurstaki cry1Ac* sequence, under the control of the cauliflower mosaic virus 35S promoter (Stewart et al., 1996). The vector used in the transformation also contained a hygromycin-selectable marker. Untransformed wildtype plants of the parent cultivar Oscar were used as controls. This combination of transgenic and control plants allows for the detection of potential effects associated with the transformation process as well as of effects associated with the transgene products themselves.

Plants were grown in 1 l pots in a glasshouse set at 20°C and a minimum day length of 14 h. Plants were checked daily and any flower buds removed, as required by statutory biosafety rules.

Cry1Ac expression in Bt oilseed rape leaves was quantified by ELISA as described previously (Schuler et al., 2001) for every batch of plants used, and expression ranged between 0.003 and 0.02% of total protein. In addition to the biochemical analyses, leaf material from every batch of plants was tested in a bioassay with susceptible third instar *P. xylostella* larvae to detect any decrease in the level of plant resistance and the plants always caused 100% mortality.

### *Insects*

This study used a normal susceptible *P. xylostella* strain as well as a laboratory-selected *P. xylostella* strain (NO-QA) with an unusually high level of resistance, which allowed the larvae to survive on Bt brassicas. The susceptible strain originated in the Philippines and had been cultured at Rothamsted for over 10 years without exposure to Bt or other insecticides (Furlong & Pell, 1996). The resistant NO-QA strain originated from Hawaii, where microbial Bt formulations are widely used, and had been further selected in the laboratory with a microbial Bt ssp. *kurstaki* formulation for high levels of resistance to Bt toxins (Tabashnik et al., 1997). Both strains were maintained on untransformed oilseed rape plants (cultivar Falcon) at 22 ± 3°C.

A culture of the solitary larval endoparasitoid *C. plutellae* was maintained in the laboratory on the susceptible *P. xylostella* strain (Potting et al., 1999). Adult parasitoid females used in experiments emerged together with males in large culture cages where they were provided with honey solution.

An oviposition experience increases the response of adult *C. plutellae* females to volatile cues (Potting et al., 1999) and females were therefore allowed to oviposit in three to five susceptible host larvae on a host-damaged wildtype leaf 1–2 h prior to release in the wind tunnel. Females used in the wind tunnel experiments were 3–6 days old. Parasitoids released in the field simulator cages were naïve 1–4 days old *C. plutellae* females, that is, they had no previous oviposition experience.

### *Wind tunnel experiments*

The flight responses of adult female *C. plutellae* to oilseed rape leaves were investigated in a wind tunnel (90 cm × 30 cm × 30 cm) as described by Potting et al. (1999). The environmental conditions in the room were 22 ± 1°C, 60 ± 10 r.h. Parasitoids were released downwind at a distance of 70 cm from two leaves, which were placed 15 cm apart. Only young leaves were used with a length of ca. 10 cm and a width of ca. 6 cm. Oriented flights that ended in landings on one of the two leaves, within 5 min after initiation of flight, were recorded as a choice.

A series of six dual choice tests were done in the wind tunnel to investigate if *C. plutellae* females distinguish between (a) Bt and wildtype oilseed rape plants and (b) plants damaged by either susceptible or Bt-resistant *P. xylostella* larvae. A study with wildtype oilseed rape showed that *C. plutellae* predominantly uses plant-derived stimuli in its in-flight host searching behaviour and the presence of hosts is not essential (Potting et al., 1999). However, host-damaged leaves of wildtype plants are more attractive to *C. plutellae* females than undamaged wildtype leaves, and artificially damaged leaves are as attractive as host-damaged leaves (Potting et al., 1999). The present study therefore incorporated both host-damaged and artificially damaged leaves.

Two whole leaves were used in each choice test. To obtain leaves that did not wilt during the wind tunnel experiments, excised leaves were kept for 24–48 h prior to the test in a vial with water and only turgid leaves were used for the tests. Host-damaged leaves were obtained by allowing two second to third instar *P. xylostella* larvae to feed on each leaf for 16–20 h, after which the hosts were removed. The damage caused by the larvae was quantified by measuring the area of feeding holes with the aid of graph paper. Artificially damaged leaves were obtained by punching five 2 mm holes in each leaf 1–2 h prior to the test.

The first test provided the parasitoids with a choice of Bt and wildtype leaves damaged by Bt-susceptible *P. xylostella* larvae. In the second test the parasitoids were offered a choice between two Bt leaves damaged by either Bt-resistant or Bt-susceptible hosts. The third test provided the parasitoids with a choice of a Bt and a wildtype leaf damaged by Bt-resistant hosts. In the fourth and fifth tests the response of parasitoids to artificially damaged Bt or wildtype leaves, respectively, was investigated. The final wind tunnel test provided a choice between two artificially damaged leaves of the two plant types. Tests 4 and 5 used 20 parasitoid females each and the remaining tests used 40 females per test. For each dual choice test three to four sets of leaves were used over two to three experimental days.

*Population scale experiments in field simulator cages*

Four field simulator cages measuring 1.7 m × 1.2 m × 1.0 m were used as described previously (Schuler et al., 2001). Experiments were conducted using a light:dark regime of 16 h:8 h, a room temperature of 21 ± 1°C resulting in 26 ± 4°C inside the cages and ambient relative humidity conditions. Plants were 35–39 days old at the start of the experiment.

Three experiments were conducted in the field simulator cages to compare the level of parasitism on Bt and wildtype plants in conditions under which parasitoid females can choose between host populations on either plant type. The experiments were conducted with Bt-resistant *P. xylostella* larvae since Bt-susceptible *P. xylostella* larvae do not survive on Bt oilseed rape plants and therefore do not allow *C. plutellae* larvae to complete their development (Schuler et al., 1999).

For each experiment four wildtype and four Bt Oscar plants were placed together in each cage as described previously (Schuler et al., 2001) and each plant was infested with 100 *P. xylostella* eggs. Egg viability was assessed separately by monitoring the hatch rate of 200–240 surplus eggs of the same egg batch. These hatch rates ranged between 91 and 99%. Plants were placed in trays filled with water to prevent *P. xylostella* larvae from moving between plants. In the second and third experiment, plants were also prevented from touching the sides of the cage since, in the first experiment, some mature unparasitised *P. xylostella* larvae had moved off the plant to pupate on the cage walls.

In each experiment, four mated adult female parasitoids were released in the centre of two of the four cages. *C. plutellae* preferentially attacks second and third instar diamondback moth larvae (Kawaguchi & Tanaka, 1999). Thus, in experiment 1, the release was timed to coincide with the majority of *P. xylostella* larvae being in their early third instar (9 days after infestation with *P. xylostella* eggs), as far as could be judged without disturbing the plants. In experiment 2, *P. xylostella* larvae developed somewhat faster, and when the parasitoids were released 8 days after infestation with *P. xylostella* eggs, most hosts appeared to be already in their late third instar and some fourth instar larvae were also present. In experiment 3, the release of parasitoids was timed to mimic the situation in experiment 2. In each experiment, the remaining two cages were used as controls, with no parasitoids added.

To allow an accurate assessment of numbers of parasitised and unparasitised hosts, plants were removed from the cages when the majority of unparasitised hosts had pupated but before adult moths emerged (5–6 days after parasitoid release). All insects on the plants were counted. In experiment 1 some hosts pupated on the side of the cage in the vicinity of where leaves touched the cage. These pupae were attributed

to the nearest host plant and included in the analysis. Since hosts parasitised by *C. plutellae* do not pupate, hosts that had pupated were recorded as unparasitised and destroyed. Host larvae that had not pupated (that were either parasitised or had not yet pupated) were transferred to Petri dishes containing a 7 cm diameter leaf disk cut from the same plant and maintained at 21 ± 1°C to record further host pupation, emergence of parasitoid larvae from hosts, as well as emergence of adult parasitoids from their pupal cocoons.

*Effect of Bt oilseed rape on the developmental rate of Bt-resistant hosts*

To assist with the interpretation of population cage experiments, a Petri dish bioassay was conducted to investigate if the developmental rate of the Bt-resistant *P. xylostella* strain was slower on Bt oilseed rape compared to wildtype oilseed rape. Neonate *P. xylostella* were placed on leaf discs (4 cm diameter) of either Bt oilseed rape or wildtype oilseed rape in filter paper-lined Petri dishes (9 cm diameter) and reared at 25 ± 1°C until adult emergence. Ten larvae were used per Petri dish and each treatment replicated 10 times. Leaf disks were replaced every 3 days with a fresh leaf disk (7 cm diameter) of the appropriate plant type until larvae had pupated. Parameters recorded included larval weight, mortality, time to pupation and time to adult emergence.

*Statistical analysis*

The observed percentage of parasitoids choosing to land on a leaf in the wind tunnel choice tests was compared against a null hypothesis of random choice. Differences in feeding damage to leaves used in the wind tunnel choice tests 1–3 were tested using a Student's *t*-test.

In the first population scale experiment the proportions of hosts recovered, hosts parasitised and adults emerging from parasitised hosts were analysed using a generalised linear mixed model (GLMM). In population scale experiments 2 and 3 the proportion of hosts recovered was analysed using a GLMM. Recovered hosts that were parasitised and adult parasitoids emerging from parasitised hosts were analysed using a generalised linear model (GLM). The presence of overdispersion in the parasitised hosts data was accounted for using Williams' procedure. Numbers of parasitoid adults and pupae were analysed using analysis of variance (ANOVA).

For the developmental bioassay the proportions of pupating larvae and emerging adults were analysed using logistic regression. Total mortality at day 13 was analysed using a test for the difference between two proportions. Untransformed mean larval weights per replicate at day 5 were compared using ANOVA.

## Results

*Wind tunnel choice tests*

Susceptible *P. xylostella* larvae caused significantly less feeding damage to Bt leaves than to wildtype leaves ($t_4 = 5.64$, $p = 0.005$; Figure 1(a)). When

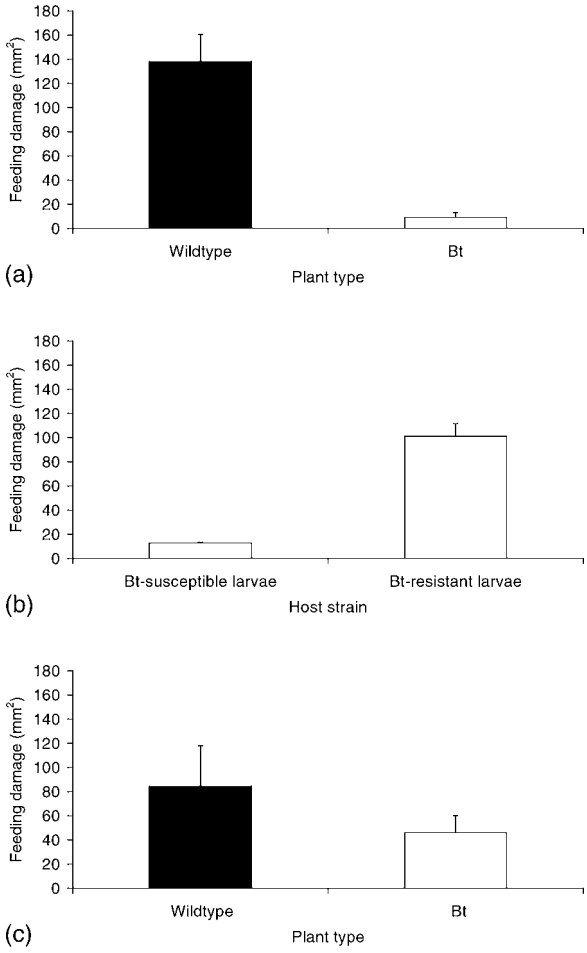


*Figure 1.* Feeding damage caused by *P. xylostella* larvae to oilseed rape leaves, which were subsequently offered to *C. plutellae* females in wind tunnel choice tests (mean ± SEM) (a and b, $n = 3$; c, $n = 4$). (a) Bt-susceptible *P. xylostella* larvae (test 1). (b) Both *P. xylostella* strains on Bt leaves (test 2). (c) Bt-resistant *P. xylostella* larvae (test 3).

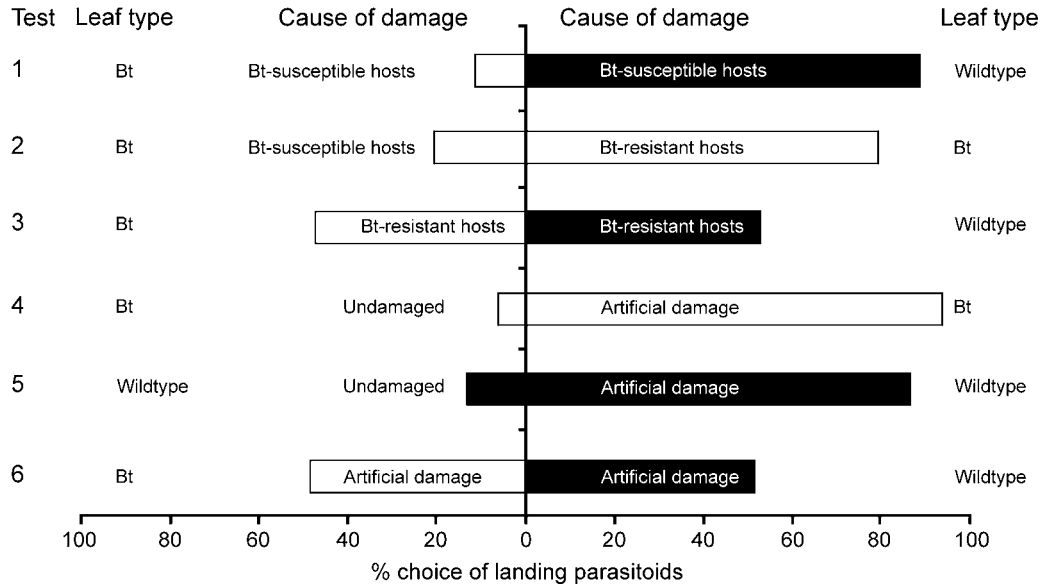


*Figure 2.* The response of *C. plutellae* to oilseed rape leaves in dual choice tests in a wind tunnel. The bars indicate the percentage of parasitoids attracted to and landing on Bt (white) and wildtype (black) oilseed rape leaves. In tests 1–3 damage was inflicted by feeding Bt-resistant or susceptible *P. xylostella* larvae. In tests 4–6 damage was artificially inflicted by punching holes in each leaf. (1) $n = 35$ landings on test leaves, $p < 0.001$; (2) $n = 34$, $p < 0.001$; (3) $n = 36$, $p = 0.43$; (4) $n = 16$, $p < 0.001$; (5) $n = 15$, $p = 0.004$; (6) $n = 31$, $p = 0.5$.

these leaves were placed in the wind tunnel, 31 out of 40 *C. plutellae* females landed on the wildtype leaf and only 4 landed on the Bt leaf ($p < 0.001$; Figure 2; test 1; five females did not respond). Susceptible larvae caused significantly less feeding damage to Bt leaves than Bt-resistant larvae ($t_4 = -8.52$, $p = 0.001$; Figure 1(b)). When these leaves were offered to the parasitoid females, 27 out of 40 parasitoids flew to the Bt leaves damaged by resistant larvae, with only 7 choosing Bt leaves damaged by susceptible larvae, a difference that was highly significant ($p < 0.001$; Figure 2; test 2). No significant difference was found in feeding damage by resistant larvae on Bt and wildtype plants ($t_6 = 1.05$, $p = 0.33$; Figure 1(c)), and the parasitoids did not distinguish between these two treatments in test 3. Of the 40 parasitoids tested, almost equal numbers landed on each plant type (Bt leaves, 19 females; wildtype leaves, 17 females; $p = 0.4$; Figure 2; test 3); while four did not respond.

Parasitoids strongly preferred artificially damaged leaves to undamaged leaves: of the 20 parasitoids tested in the fourth wind tunnel test, 15 landed on the artificially damaged Bt leaf compared to only one parasitoid that landed on an undamaged Bt leaf ($p < 0.001$; Figure 2; test 4). A very similar response was obtained in test 5 with wildtype leaves: of the 20 parasitoids tested 13 chose the artificially damaged leaf and two the undamaged leaf ($p = 0.004$; Figure 2; test 5). Parasitoids did not discriminate between wildtype and Bt leaves if leaves were artificially damaged to the same degree: of 50 parasitoids tested 15 landed on Bt leaves and 16 landed on wildtype leaves ($p = 0.50$; Figure 2; test 6).

### *Population scale experiments*

There was no significant difference between Bt and wildtype plants in the level of parasitism of Bt-resistant *P. xylostella* by *C. plutellae* in experiment 1 (Table 1). In contrast, experiment 2 indicated a higher level of parasitism by *C. plutellae* on Bt plants ($p < 0.01$), whilst experiment 3, aimed at releasing parasitoids when hosts had reached a similar size to those in experiment 2, showed no significant difference between wildtype plants and Bt plants (Table 1). However, levels of parasitism were generally lower in experiment 3 than in experiments 1 and 2 (Table 1).

Over 93% of adult parasitoids emerged successfully from their cocoons in each experiment and there was no significant effect of plant type on adult parasitoid emergence (Table 1). Bt plants had no significant effect ($p > 0.05$) on the number of parasitoid adults that emerged per plant in experiments 1 and 3 but there was some evidence of a higher number of parasitoids on Bt plants ($p = 0.057$; Table 1) in experiment 2 (in which percentage parasitism was also higher).

*Table 1.* The effect of Bt oilseed rape on parasitism of Bt-resistant diamondback moth larvae by *C. plutellae* in field simulator cage experiments

| Experiment | Plant line | % parasitism (mean ± SEM)[a] | % adult parasitoid emergence from cocoons (mean ± SEM)[b] | Mean log transformed number of parasitoid adults produced per plant (mean ± SEM, $n = 8$)[c] |
|---|---|---|---|---|
| 1 | Bt | 57.9 ± 2.6 | 97.0 ± 1.2 | 1.37 ± 0.05 |
| | Wildtype | 53.7 ± 2.4 | 96.1 ± 1.3 | 1.43 ± 0.05 |
| 2 | Bt | 62.2 ± 4.5 | 93.0 ± 1.9 | 1.32 ± 0.05 |
| | Wildtype | 38.6 ± 4.4 | 96.3 ± 1.6 | 1.16 ± 0.05 |
| 3 | Bt | 16.6 ± 2.8 | 100.0 ± 0.0 | 0.84 ± 0.11 |
| | Wildtype | 11.0 ± 2.4 | 97.7 ± 2.3 | 0.59 ± 0.11 |

[a] Experiment 1, $\chi^2_1 = 0.6$, $p > 0.05$; experiment 2, $\chi^2_1 = 13.29$, $p < 0.001$; experiment 3, $\chi^2_1 = 2.33$, $p > 0.05$.
[b] Experiment 1, $\chi^2_1 = 0.2$, $p > 0.05$; experiment 2, $\chi^2_1 = 1.80$; $p > 0.05$; experiment 3, not analysed since all except one adult emerged.
[c] Experiment 1, $F_{1,13} = 0.66$, $p = 0.431$; experiment 2, $F_{1,13} = 4.37$; $p = 0.057$; experiment 3, $F_{1,13} = 2.58$, $p = 0.132$.

*Table 2.* Survival of Bt-resistant diamondback moth larvae on plants in field simulator cages in the presence or absence of adult female *C. plutellae*[a]

| Experiment | Plant line | % total hosts recovered per plant (larvae and pupae) (mean ± SEM) | | Number of host pupae per plant (mean ± SEM) $n = 8$ | |
|---|---|---|---|---|---|
| | | Parasitoids added | No parasitoids added | Parasitoids added | No parasitoids added |
| 1 | Bt | 44.4 ± 4.8 | 63.1 ± 4.6 | 18.4 ± 3.9 | 63.1 ± 3.9 |
| | Wildtype | 55.9 ± 4.8 | 63.4 ± 4.6 | 25.5 ± 3.9 | 63.4 ± 3.9 |
| 2 | Bt | 43.8 ± 3.6 | 61.5 ± 3.6 | 15.1 ± 2.8 | 61.2 ± 2.8 |
| | Wildtype | 45.5 ± 3.7 | 61.4 ± 3.6 | 25.5 ± 2.8 | 61.2 ± 2.8 |
| 3 | Bt | 49.8 ± 4.2 | 59.6 ± 4.2 | 41.2 ± 1.5 | 59.6 ± 1.5 |
| | Wildtype | 48.8 ± 4.2 | 64.5 ± 4.1 | 43.1 ± 1.5 | 64.4 ± 1.5 |

[a] 100 diamondback moth eggs were placed on each plant.

The total numbers of hosts (parasitised and unparasitised) recovered per plant were similar in the three experiments (Table 2). The presence of *C. plutellae* females significantly reduced the number of hosts retrieved per plant but the plant type had no effect and there was no significant interaction between plant type and the presence of parasitoids (Table 3).

The presence of parasitoids significantly reduced the number of host pupae per plant in the first two experiments ($p = 0.009$, 0.003, respectively) but not in the third experiment, probably due to generally low levels of parasitism (Tables 2 and 3). There was no significant effect of plant type on numbers of pupae per plant in experiments 1 and 3. In experiment 2 there was some evidence for better *P. xylostella* control on Bt plants ($p = 0.077$) as well as for an interaction between plant type and parasitoid treatment ($p = 0.077$) (Tables 2 and 3), which is in line with the higher level of parasitism observed on Bt plants in this experiment (Table 1).

### *Effect of Bt oilseed rape on the developmental rate of Bt-resistant hosts*

The second population scale experiment gave a higher level of parasitism of *P. xylostella* on Bt plants. One

*Table 3.* Statistical analyses of the survival of Bt-resistant diamondback moth larvae on plants in field simulator cages in the presence or absence of adult female *C. plutellae*

| Experiment | Comparisons of total hosts recovered per plant | | | Comparisons of number of host pupae per plant | | |
|---|---|---|---|---|---|---|
| | Parasitoids (present/absent) | Plant type | Interaction | Parasitoids (present/absent) | Plant type | Interaction |
| 1 | $\chi^2_1 = 7.4$<br>$p < 0.01$ | $\chi^2_1 = 1.5$<br>$p > 0.05$ | $\chi^2_1 = 1.3$<br>$p > 0.05$ | $F_{1,2} = 108.34$<br>$p = 0.009$ | $F_{1,26} = 0.90$<br>$p = 0.352$ | $F_{1,2} = 0.78$<br>$p = 0.385$ |
| 2 | $\chi^2_1 = 19.8$<br>$p < 0.001$ | $\chi^2_1 = 0.0$<br>$p > 0.05$ | $\chi^2_1 = 0.1$<br>$p > 0.05$ | $F_{1,2} = 330.78$<br>$p = 0.003$ | $F_{1,26} = 3.40$<br>$p = 0.077$ | $F_{1,26} = 3.40$<br>$p = 0.077$ |
| 3 | $\chi^2_1 = 8.0$<br>$p = 0.005$ | $\chi^2_1 = 0.2$<br>$p > 0.05$ | $\chi^2_1 = 0.5$<br>$p > 0.05$ | $F_{1,2} = 13.04$<br>$p = 0.069$ | $F_{1,26} = 0.74$<br>$p = 0.397$ | $F_{1,26} = 0.14$<br>$p = 0.711$ |

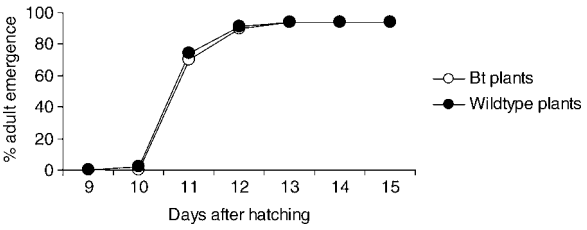


*Figure 3.* Developmental time from hatchling to adult of the Bt-resistant *P. xylostella* NO-QA strain on Bt oilseed rape compared to wildtype oilseed rape.

possible explanation for this result is that Bt plants might sublethally affect *P. xylostella* larvae, resulting in a slower growth rate. *C. plutellae* females are more successful in attacking small *P. xylostella* larvae than large fourth instar larvae and a slower development on Bt plants would therefore extend the 'window of opportunity' for parasitoid attack. However, there was no evidence for any sublethal effects of Bt plants on *P. xylostella* larvae. Larval weight 5 days after hatching was not significantly different on the two plant types ($F_{1,18} = 0.02$, $p = 0.878$). There was also no delay in pupation ($\chi^2_1 = 0.16$, $p = 0.690$) or adult emergence ($F_{1,18} = 0.05$, $p = 0.828$; Figure 3) levels on Bt plants. Mortality 13 days after hatching was 4% on both Bt and wildtype oilseed rape ($z = 0.01$, $p = 0.988$).

## Discussion

Volatiles released by plants are important long-range cues for parasitoids foraging for hosts (Vet & Dicke, 1992; Potting et al., 1999). Wind tunnel choice tests demonstrated that adult *C. plutellae* females were more attracted in-flight to wildtype leaves damaged by Bt-susceptible hosts than to transgenic leaves damaged by such larvae. This preference of the females is advantageous to the parasitoid since Bt-susceptible *P. xylostella* larvae do not survive for long enough on Bt oilseed rape plants to allow the parasitoid larvae to complete their development (Schuler et al., 1999). Thus, this behaviour avoids wasting resources on unsuitable hosts. The long-range preference of *C. plutellae* females for wildtype leaves is due to the lower level of leaf damage caused by susceptible host larvae feeding on the transgenic plants. Feeding damage by the host leads to increased amounts of volatiles being released (Agelopolous & Keller, 1994), which act as foraging stimuli for the parasitoid, but ingestion of Bt δ-endotoxins, such as Cry1Ac, cause cessation of feeding and gut paralysis in susceptible lepidopteran larvae (Glare & O'Callaghan, 2000), resulting in very limited leaf consumption by Bt-susceptible *P. xylostella* larvae on the transgenic plants.

In the wind tunnel *C. plutellae* females did not distinguish in-flight between transgenic and wildtype leaves that were damaged to the same degree, either artificially or through feeding by Bt-resistant larvae. There was therefore no indication that the transformation process had caused changes in the volatile profile of the plant. The parasitoids did discriminate in-flight between transgenic leaves damaged by Bt-resistant or Bt-susceptible *P. xylostella* larvae. Only a small proportion of females chose to land on transgenic leaves damaged by Bt-susceptible hosts, while transgenic leaves damaged by Bt-resistant hosts were highly

attractive to the parasitoids. *C. plutellae* larvae complete their development normally in Bt-resistant hosts on Bt oilseed rape plants (Schuler et al., 1999). This stronger attraction of *C. plutellae* to transgenic crops damaged by Bt-resistant hosts could assist with constraining the spread of genes for Bt resistance in *P. xylostella* populations. However, the preference of *C. plutellae* for wildtype plants over transgenic plants when damaged by susceptible hosts could also result in a reluctance to move from non-Bt refuges into a Bt crop. Further information on the foraging ranges of parasitoids such as *C. plutellae* would be desirable to model in more detail their impact on the development of Bt-resistant pest populations.

The wind tunnel choice tests were performed with host-damaged leaves rather than the complete plant–host-complex since the presence of feeding host larvae is not necessary for long-range attraction of *C. plutellae* (Potting et al., 1999). However, host habitat location is only the first step necessary for successful parasitism (Vinson, 1984, 1985). The population scale experiments incorporated further steps such as host location, host acceptance and host suitability through offering female parasitoids a choice between resistant hosts feeding on whole transgenic and wildtype plants. These experiments also incorporated host defence behaviour. *C. plutellae* was as effective in controlling resistant *P. xylostella* on transgenic as on wildtype plants. Equal or higher numbers of parasitoid adults emerged per transgenic plant as per wildtype plant. Population scale experiments with the aphid parasitoid *D. rapae* similarly found no negative effects of Bt oilseed rape on the ability of *D. rapae* to control aphid populations (Schuler et al., 2001).

A higher level of parasitism was observed on the transgenic plant in one of the population scale experiments. One mechanism often suggested to explain such cases of synergism is that slower larval development of a pest on resistant plants extends the period of time during which a parasitoid can attack (Johnson & Gould, 1992; Bell et al., 2001). However, the Bt-resistant *P. xylostella* used in this study did not develop slower on the transgenic oilseed rape plants. Similarly, a study with a Bt-resistant *P. xylostella* strain from Florida found no reduction in weight gain of larvae feeding on Bt broccoli (Tang et al., 1999). The increase in parasitism observed on Bt oilseed rape plants was therefore not due to an extended window of opportunity for parasitoid attack. It is unclear why this effect was only apparent in one of the three field simulator experiments. The observed difference was nonetheless highly significant and merits further investigation.

The survival of populations of specialist natural enemies of *P. xylostella* in the vicinity of a Bt brassica crop will largely depend on the availability of hosts either on untransformed or on weedy brassicas nearby. The availability of such refuges for *P. xylostella* populations is likely to vary from region to region and would have to be assessed for each location separately when an introduction of a Bt brassica crop is considered. Simulations by Chilcutt and Tabashnik (1999) of the integration of microbial Bt sprays with *C. plutellae* suggested that under certain conditions *C. plutellae* can slow the evolution of Bt resistance, but without pest immigration or a Bt-free refuge *C. plutellae* could be eliminated from the system. The creation of such refuges is already a component of current resistance management plans for Bt cotton and Bt maize (Roush, 1997; Carriere & Tabashnik, 2001). Their main purpose is to allow Bt-susceptible moths to survive and mate with any resistant individuals present. However, refuges may also help with maintaining populations of specialist parasitoids in the vicinity of the Bt crop.

Effects of transgenic crops on natural enemies have to be judged in comparison with other control measures. Any action taken by farmers to protect their crop from pest insects will reduce host availability for natural enemies. Many synthetic insecticides used for diamondback moth control are toxic on contact not only for the pest but also for parasitoids. Microbial Bt sprays are widely used against *P. xylostella*, often as part of integrated pest management programmes, and they also cause host mortality within a few days, not allowing *C. plutellae* larvae to complete their development (Talekar & Shelton, 1993; Chilcutt & Tabashnik, 1997; Sivapragasam et al., 1997). However, Bt brassicas are likely to be more effective in controlling *P. xylostella* populations than Bt sprays since exposure to the toxin is more targeted and the toxin is expressed throughout the life of the plant (Metz et al., 1995b).

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