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ORIGINAL PAPER

Prey-mediated effects of transgenic canola on a beneficial, non-target, carabid beetle

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Abstract Transgenic plants producing insecticidal proteins from *Bacillus thuringiensis* (Bt) can control some major insect pests and reduce reliance on sprayed insecticides. However, large scale adoption of this technology has raised concerns about potential negative effects, including evolution of pest resistance to Bt toxins, transgene flow from Bt crops to other plants, and harm to non-target beneficial organisms. Furthermore, concern has also been expressed over the effects this technology may have on biodiversity in general. Ecologically relevant risk assessment is therefore required (Risk = Hazard × Exposure). Transgenic plants that produce Bt toxins to kill insect pests could harm beneficial predators. This might occur directly by transmission of toxin via prey, or indirectly by toxin-induced reduction in prey quality (Hazard). To test these hypotheses, we determined the effects of Bt-producing canola on a predatory ground beetle (*Pterostichus madidus*) fed larvae of diamondback moth (*Plutella xylostella*) that were either susceptible or resistant to the Bt toxin. Survival, weight gain, and adult reproductive fitness did not differ between beetles fed prey reared on Bt-producing plants and those fed prey from control plants. Furthermore, while Bt-resistant prey was shown to deliver high levels of toxin to the beetle when they were consumed, no significant impact upon the beetle was observed. Subsequent investigation showed that in choice tests (Exposure), starved and partially satiated female beetles avoided Bt-fed susceptible prey, but not Bt-fed resistant prey. However, in the rare cases when starved females initially selected Bt-fed susceptible prey, they rapidly rejected them after beginning to feed. This prey type was shown to provide sufficient nutrition to support reproduction in the bioassay suggesting that Bt-fed susceptible prey is acceptable in the absence of alternative prey, however adults possess a discrimination ability based on prey quality. These results suggest that the direct effects of Bt-producing canola on predator life history was minimal, and that predators' behavioural preferences may mitigate negative indirect effects of reduced quality of prey caused by consumption of Bt-producing plants. The results presented here therefore suggest that cultivation of Bt canola

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may lead to conservation of non-target predatory and scavenging organisms beneficial in pest control, such as carabids, and may therefore provide more sustainable agricultural systems than current practices. In addition, minimal impacts on beneficial carabids in agro-ecosystems suggest that Bt canola crops are likely to be compatible with integrated pest management (IPM) systems.

Keywords *Bacillus thuringiensis* · Beneficial predators · Carabid · Non-target · Resistance · Tritrophic interactions

Introduction

Transgenic plants producing insecticidal proteins from *Bacillus thuringiensis* (Bt) can control some major insect pests and reduce reliance on sprayed insecticides (Phipps and Park 2002, Shelton et al. 2002). In 2005 the global area of GM crops exceeded 90 million hectares with a global market of $6.5 billion, of which 25% was attributed to Bt expressing crops (James 2005). This large scale adoption has raised concerns over potential negative effects of Bt crops, including evolution of pest resistance to Bt toxins, transgene flow from Bt crops to other plants, and harm to beneficial organisms (Gould 1998; Wolfenbarger and Phifer 2000, Groot and Dicke, 2002, Ellstrand 2003). Bt crops kill a high proportion of the populations of some highly susceptible target pests (Tabashnik et al. 2000, 2003), yet non-target insects and some less susceptible target insects may consume Bt crops and survive, thereby exposing their predators and parasitoids to the Bt toxin. In addition, several generalist predators, beneficial in pest control, may also be scavengers and become exposed to Bt via consumption of sick poisoned prey.

Although most studies report that Bt crops do not harm predators and other beneficial organisms (Ferry et al. 2003a), a few studies have shown negative effects (Hilbeck et al. 1999; Losey et al. 1999; Meier et al. 2001). Hilbeck et al. (1999) reported prey-mediated negative effects for the predatory lacewing; however, Dutton et al. (2002) subsequently demonstrated that these negative effects on the predator were indirect in that they were due to reduction in prey quality as a consequence of ingesting the toxin, rather than by direct effects of the Bt toxin itself following consumption of prey that had fed on Bt expressing plants. Romeis et al. (2004) and Rodrigo-Simón et al. (2006) further demonstrate that the Bt toxin fails to bind to the gut membrane of the predator, a pre-requisite for direct toxicity.

Previous studies have examined direct and indirect effects of Bt toxins in model tri-trophic systems with cruciferous plants, the herbivore *Plutella xylostella* (diamondback moth), and the parasitoid *Cotesia plutellae* (Chilcutt and Tabashnik 1997a, b; 1999a, b; Schuler et al. 1999, 2003). Chilcutt and Tabashnik (1997a, b; 1999a) used *Brassica oleraceae* (cabbage) leaves that were either untreated or treated externally with a commercial formulation of Bt toxins called Dipel. Schuler et al. (1999, 2003) used two types of *Brassica napus* (oilseed rape/canola), a conventional variety and a transgenic variety that expressed Bt toxin Cry1Ac. The herbivore studied (*P. xylostella*), a global pest of crucifers, is the only insect with documented field-evolved resistance to Bt toxins (Ferre and Van Rie 2002; Tabashnik et al. 2003). Comparison of parasitoid responses to susceptible vs. resistant *P. xylostella* larvae fed plant material with or without Bt toxins enabled discrimination between direct and indirect effects. When Bt toxin Cry1Ac was delivered via transgenic canola or Dipel treated cabbage leaves, the parasitoid developed successfully on resistant *P. xylostella* larvae, but not on susceptible *P. xylostella* larvae. In effect, since susceptible *P. xylostella* larvae could not survive when Bt toxins were included in their diet, the parasitoid was deprived of its food source. Conversely, resistant *P. xylostella* were not deleteriously effected by Bt toxins, enabling parasitoid development.

Carabid beetles are widespread, ground-dwelling predators and scavengers that are important natural enemies of agricultural pests, including various species of Lepidoptera (Lövei and Sunderland 1996). Carabids are often used to monitor environmental changes, including the impact of pesticides (Volkmar et al. 2002), and thus are of high priority in risk assessment studies.

Although Bt toxin Cry1Ac is not likely to have a direct toxic effect on carabids (due to high specificity), indirect effects could occur because both food quality and quantity affect carabid fecundity (Wallin et al. 1992, Ferry et al. 2005). We focused on *P. madidus* since it is one of the most common British carabids occurring in agricultural landscapes. Meissle et al. (2005) report effects on the mortality and development of predatory carabid larvae (*Poecilus cupreus*) when fed Bt Cry1Ab containing prey, however direct and indirect effects could not be distinguished. We focus here on direct and indirect prey-mediated effects of Bt toxins on a predatory carabid beetle, *P. madidus* via feeding on Bt resistant and susceptible prey.

Our model system used canola (oilseed rape) as the plant, larvae of *P. xylostella* as the herbivorous prey, and *P. madidus* as the predator. We compared insect responses to untreated, conventional canola with responses to transgenic canola that expresses and accumulates Bt toxin Cry1Ac (Stewart et al. 1996). We compared effects on the predator of consuming (a) susceptible prey fed control canola (Control(S)), (b) resistant prey fed control canola (Control(R)), (c) susceptible prey fed Bt canola (Bt(S)), and (d) resistant prey fed Bt canola (Bt(R)). We also tested the hypothesis that the predator avoids prey poisoned by Bt toxin. This is thus the first study of its type to discriminate between direct and indirect prey quality effects on a generalist predator.

Materials and methods

Materials

The Bt Cry1Ab/1Ac DAS ELISA kit was obtained from Agdia Inc. USA.

Homozygous transgenic canola (oilseed rape; *Brassica napus* (L.) cv. Oscar, line O52) was generated as previously described (Stewart et al. 1996). Zygotic hypocotyls were transformed with a truncated synthetic *Bt* Cry1Ac construct under the control of the cauliflower mosaic virus 35S promoter using *Agrobacterium tumefaciens*-mediated transformation. Feeding experiments were performed using T3 transgenics and the non-transformed cv Oscar as the control. All plant lines were grown concurrently under the same controlled environmental conditions: 16 h day/8 h night at a temperature of 20°C ± 2°C. All transgenic plants were sampled (leaves) at 8 weeks for expression of the protein and used in subsequent bioassays.

Stocks of diamondback moth (*P. xylostella*) from continuous culture over many generations (*Bt* susceptible) and strain NO-QA (Bt *kurstaki* resistant) (Tabashnik et al. 1990) were reared on Chinese cabbage plants under controlled environmental conditions (20°C ± 2°C, under a L16:D8 light regime). NO-QA was subjected to two generations of selection on Bt Cry1Ac expressing canola prior to entry into bioassays.

Pterostichus madidus adults were collected within the grounds of the University field station (Close-House, Heddon on the Wall) in June and July 2001 (this coincided with the emergence of new adults from pupae (Luff 1973). Any overwintered adults likely to be going through a second maturation cycle of the ovaries were identified on the basis of mandibe wear and excluded from the trials). Adult beetles were maintained individually in controlled environment incubators (12°C ± 2°C, L16:D8 light regime) and fed larvae of *Calliphora* sp. until August 2001 (the main reproductive period), when beetles were transferred to a soil substrate and mated prior to trials.

Determination of transgene expression levels in canola

Leaf samples were taken at random from both transgenic plants and non-transformed control plants, flash-frozen in liquid nitrogen, ground to a fine powder and extracted in 50 mM Na_2CO_3 buffer, pH 10.5 with the addition of 1% (v/v) phenyl-methylsulfonyl fluoride (PMSF) (36 mg/ml in ethanol) overnight at 4 °C with shaking. Extracts were centrifuged at 10,000*g* for 15 min and total soluble protein of the supernatants was estimated by Bradford assay, with bovine serum albumin (BSA) as a standard (Bradford 1976). Samples were subsequently diluted in milk enhanced sample extract buffer (MEB) (phosphate buffered saline (PBS) with 0.4% non-fat

dried milk and 0.5% Tween-20) to give a final protein concentration of 2 μg ml^{-1}. Cry1Ac toxin present in leaf samples was detected by ELISA as per the manufacturers instructions. In brief, 100 μl of either plant extract, or purified Cry1Ac toxin standards (to give final concentrations from 0.125 to 32 ng of Cry1Ac in MEB sample extract buffer) were incubated for 3 h in ELISA plates coated with anti-Cry1Ac antibody in an airtight container with 100 μl of alkaline phosphatase enzyme conjugate. Unbound protein was removed by washing (×6) with phosphate-buffered saline (PBS) + 0.01% Tween 20 (v/v) (PBST). Wells were again washed and then the assay was developed by the addition of 200 μl *p*-nitrophenyl phosphate (PNP) substrate solution and absorbance read at 405 nm in a microtitre plate reader. Levels of Cry1Ac were determined from the Cry1Ac calibration curve. Each sample was assayed in triplicate. All incubations were performed at room temperature.

Detection of Bt toxin Cry1Ac in *P. xylostella* gut tissues

The whole gut from 15 final instar *P. xylostella* larvae (susceptible and resistant strains), previously fed either transgenic Cry1Ac expressing plants or non-transformed control plants for a minimum of 24 h, were dissected into 50 mM Na_2CO_3 buffer, pH 10.5, with the addition of 1% (v/v) PMSF (36 mg/ml in ethanol), homogenised briefly and extractions performed for 1 h at 4 °C with shaking (1 gut μl^{-5}). Samples were treated as detailed above, with the following differences: insect samples were diluted in MEB to give a final protein concentration of 20 μg ml^{-1} for ELISA.

Bioassay of the effects of cry1Ac expressing canola on *P. madidus* through the tritophic interaction

Effects of cry1Ac expressing canola on the pest

Whole plant bioassays were conducted to determine the effects of cry1Ac expression in canola on both body mass change and survival of fourth (final) instar larvae of susceptible and resistant strains of the pest *P. xylostella*, following 24 h of exposure to treatments. Prior to bioassay, *P. xylostella* larvae were reared to the final instar on Chinese cabbage plants.

Effects of cry1Ac expressing canola on the predator via the pest

Effects of Cry1Ac canola on predator survival and body mass change

Mated adults of *P. madidus* were divided into four groups, designated: (a) susceptible prey fed control canola (Control(S)), (b) resistant prey fed control canola (Control(R)), (c) susceptible prey fed Bt canola (Bt(S)), and (d) resistant prey fed Bt canola (Bt(R)) and placed individually into 20 cm petri-dishes with a damp compost substrate. *P. madidus* adults were fed daily on an equal weight (ad libitum) of one group type of *P. xylostella* larvae, and unconsumed food remains removed. Prior to use as a food source for beetles, *P. xylostella* larvae were fed for a minimum period of 24 h on transgenic/control canola (sub-lethal exposure) so as to ensure that the pest/prey larvae were sufficiently dosed. Survival of *P. madidus* was monitored on a daily basis for a 30-day period. Adult weight change (measured weekly) and prey consumption (measured via the daily weight of remaining uneaten prey) was recorded throughout the trial. *P. madidus* adult weight was measured prior to entry into the trial and beetles were assigned into groups with no significant difference in start weight. $N = 20$ (control (S); Bt (R)), $N = 22$ (Bt (S); control (R)).

Effects of cry1Ac expressing canola on predator fecundity

Prior to entry into the trial, adult beetles were sexed and assigned to mating pairs. Beetles were left 72 h to ensure that females were mated. Since *P. madidus* lays most eggs in one batch, rather than continuous production over an extended laying period (Luff 1973), collection of eggs over a relatively short period of time provides a useful measure of reproductive fitness. Adult female fecundity was estimated by counts of number of eggs produced per individual. Eggs were transferred to 7 cm petri-dishes lined with damp

blotting paper and kept in controlled environment incubators in the dark at 12 ± 2 °C until hatch, counts of larvae were taken to assess egg viability, un-hatched eggs were kept for up to one month, after which point they were considered infertile (infertile and thus possibly non-mated females were removed from the analysis).

Behavioural studies

P. xylostella larvae that had been fed on either transgenic canola or non-transformed control canola for 24 hours were used in choice trials. Control fed susceptible (control (S)) larvae were used as the only control as preliminary experiments showed no significant differences between *P. madidus* selecion of control (S) and control (R) larvae (results not shown).

Choice tests

Detailed studies were conducted to assess whether the behaviour of *P. madidus* is affected by the use of Bt toxin Cry1Ac to control the prey. Choice tests were therefore designed to assess whether these predators will show any preference for (a) Bt (S) vs. Control (S) prey; (b) Bt (S) vs. Bt (R) prey or (c) Bt (R) vs. Control (S) prey. Differences in beetle searching behaviour and prey handling time were also monitored. Since *P. madidus* will consume dead prey in the field, all prey items were flash frozen after feeding and thawed prior to being offered to beetles, so as to ensure that prey behaviour was not a factor influencing beetle choice and thus scavenging behaviour could be evaluated. *P. madidus* rarely encounter live lepidopteran larvae in the field, as they do not climb to find prey. Meissle et al. (2005) have previously shown that the use of frozen prey does not change the preference of *P. cupreus* for Bt or non-Bt prey.

Adult *P. madidus* were either starved for 1 week (to ensure all gut contents were digested) or fed half a *Calliphora* sp. maggot prior to choice tests (partial satiation), and introduced into arenas of 21.5 × 12 × 7.5 cm lined with a layer of moist filter paper. Five prey items of each type were provided (larvae were assigned to size classes and only larvae from each treatment of the same size class were used in trials) and introduced into arenas at defined points to allow prey types to be distinguished. A Fluon® band was painted on the top of all arenas to prevent escape.

Arenas were placed in a controlled environment room at 12 ± 2°C under a 16-h light:8-h dark lighting regime. Behaviour was monitored using time-lapse video (Computar CTR-3024 24 hr time lapse) under infra-red light (*P. madidus* is nocturnal). Analysis was initiated at 5 pm each day and terminated at 9 am the following morning; 12 female and 12 male beetles (starved or pre-fed) were used in each analysis.

Parameters measured

(1) Prey selection,
(2) Total number of interactions made with either prey type. An interaction was defined as full contact with a prey item, with a duration >1 s,
(3) *Prey handling time*. This was defined as time in contact with prey prior to feeding, active feeding and time in contact with the prey following cessation of active feeding.

Statistical analysis

Statistical analyses were carried out separately for each prey (Bt vs. control). Comparisons between treatments for mortality were made using Kaplan–Meier Survival analysis. For data on body mass change and prey consumption, mean comparisons between treatments were made using one-way ANOVA and Student's *t*-test, respectively. For all behavioural data the non-parametric Mann–Whitney *U*-test replaced one-way ANOVA and unpaired *t*-test.

Results

Expression of cry1Ac in leaves of transgenic canola

Immunoassay by ELISA readily detected the presence of Bt toxin Cry1Ac in the vegetative tissues of the transgenic line, with levels of

transgene product accumulation ranging from 0.035 to 0.057% of total soluble protein, consistent with expression levels previously reported by Stewart et al. 1996. No Bt toxin Cry1Ac was detected in leaves of non-transformed control plants.

Effects of Bt Cry1Ac toxin on *P. xylostella* larvae and detection in gut tissue

Production of Bt Cry1Ac toxin in the vegetative tissues of transgenic canola significantly effected fourth (final) instar *P. xylostella* larvae (Fig. 1a). Bt susceptible (S) larvae stopped feeding and lost weight over a time period of 24 h (12.5% of body mass) when compared to feeding on control canola (Con(S)) ($p = 0.065$). In contrast, Bt resistant (R) larvae showed a significant weight gain following 24 h feeding on Cry1Ac expressing canola (Mann–Whitney *U*-test; p = 0.03). Both Bt (S) and Bt (R) *P. xylostella* showed significant weight gain when fed control canola (Mann–Whitney *U*-test; $p = 0.0304$ and $p = 0.03$, respectively). Mortality of Bt (S) larvae on transgenic and control plants was 100% and 0% in 72 h, respectively (results not shown).

Immunoassay by ELISA readily detected the presence of Bt toxin Cry1Ac in the guts of both Bt (S) and Bt (R) *P. xylostella* larvae fed transgenic canola (Fig. 1b); with levels of transgene product accumulation per gut 0.08 ng (±SE of 0.003 ng) and 1.6 ng (±SE of 0.05 ng) for (S) and (R) larvae, respectively. No Bt Cry1Ac toxin was detected in guts of *P. xylostella* fed non-transformed control plants.

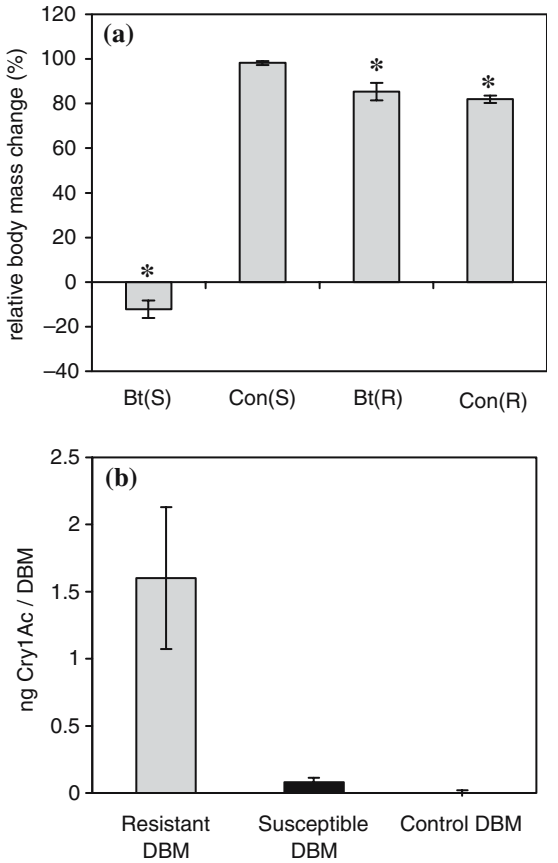

Fig. 1 (**a**) Effect of Cry1Ac expressed in vegetative tissues of transgenic canola on *P. xylostella* final instar larvae following 24 h of feeding. Larval weight change (%) relative to initial body mass was used as a measure of fitness on the treatments. Weights were compared using Mann–Whitney *U*-tests. Bars represent mean ± SE ($n = 30$). * indicates significant difference in weight at $p \leq 0.05$. Con(S) = Susceptible prey fed control canola, Con (R) = resistant prey fed control canola, Bt(S) = susceptible prey fed Bt canola, and Bt(R) = resistant prey fed Bt canola. (**b**) Presence of Cry1Ac in guts of Bt(S) and Bt(R) *P. xylostella* after feeding for 24 h on leaves of the transgenic line as detected by ELISA with anti-Cry1Ac antibodies. Values were compared to a standard curve of purified Cry1Ac standards. Control *P. xylostella* were fed leaves of untransformed canola plants. Points and bars represent mean values ± standard error for triplicated independent determinations

Effects of Bt cry1Ac expressing canola on *P. madidus* through the tritophic interaction

Effects of Bt Cry1Ac toxin on body mass change and survival of P. madidus

A short-term feeding study was carried out to investigate prey-mediated effects of transgenic canola expressing Cry1Ac on *Pterostichus madidus* when delivered via either Bt (S) or Bt (R) *Plutella xylostella.* Prey (both control and Bt treatments) was provided *ad libitum*. The results demonstrated that exposure to transgenic Cry1Ac canola dosed prey (R and S) had no significant effects on the survival of adult beetles over a thirty-day period, (Fig. 2). Kaplan–Meier Survival Analyses were performed for all groups, no significant differences between the four groups were found (Logrank (Mantel-Cox); $p = 0.73$, 0.4,

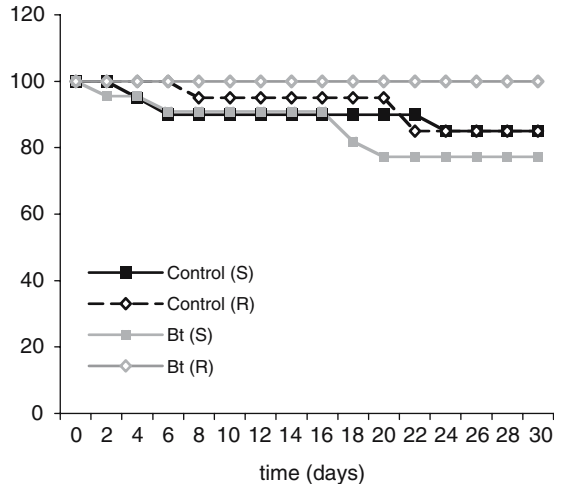

Fig. 2 Survival (%) of *P. madidus* adults over a 28 day period when fed Bt(S) or Bt(R) prey from control canola and Bt(R) or Bt(S) prey fed Cry1Ac canola. Survival was compared by Kaplan–Meir survival analysis at the end of the bioassay. (n = 20 Bt (R), Control (S) and (R); n = 22 Bt (S))

0.68 and 0.15). Although a trend towards lower survival was noted in the Bt (S) group.

Adult beetle weight was monitored at 5 time points throughout the trial (0, 7, 14, 21 and 28 days). Exposure to Bt Cry1Ac toxin via prey (S or R) fed transgenic plants resulted in no overall significant differences in adult predator weight (Table 1) (one-way ANOVA, Fishers PLSD; p = 0.11, F = 2.2). However, whilst there was an initial negative body mass change in all groups during the first week of study, subsequently all *P. madidus* adults exhibited an overall increase in body mass which was maintained for the remainder of the trial, irrespective of prey type (control(S), control(R), Bt(S) and Bt(R)).

Effects of Bt Cry1Ac toxin on fecundity of adult P. madidus

Egg production and viability were used as a measure of fecundity (Fig. 3). Adult *P. madidus* were mated prior to introduction into the trial and egg production monitored throughout the 30-day period of the bioassay. Bt Cry1Ac toxin had no significant effects on mean cumulative egg production, irrespective of prey type. Control(R), Bt(S) and Bt(R) fed beetles laid only approximately 0, 1.4 and 5.7 fewer eggs per female compared to control(S) fed beetles over the 30 day period (one-way ANOVA, Fishers PLSD; p = 0.431, F = 4.8). Subsequent viability of eggs was also evaluated and the results showed no significant effect in any of the prey groups (p = 0.645, F = 3.92) (Fig. 3).

Effects of insect-resistant transgenic plants on behaviour of the predator

Detailed studies were conducted to assess whether the predatory behaviour of *P. madidus* is influenced by transgene expression in canola via the tri-trophic interaction. Carabids are opportunistic feeders and will select the commonest prey types available; their role as scavengers has previously been underestimated and it is likely that intoxicated and dead Bt susceptible pest/prey would be available to the carabid. Arena-based choice tests were designed to assess whether these predators would show any preference for control or Cry1Ac-dosed Bt(R) or Bt(S) prey. Furthermore, they were also designed to investigate any changes in their searching behaviour and prey-handling time in response to prey contaminated with Bt Cry1Ac toxin.

Table 1 Mean body mass (mg) change of *P. madidus* adults over a 28-day period when fed Bt(S) or Bt(R) prey from control canola and Bt(R) or Bt(S) prey fed Cry1Ac canola

Treatment	Body mass change (mg) ± SE			
	Day 7	14	21	28
Control (S)	−0.3 ± 2.08	1.25 ± 1.26	0.65 ± 1.2	0.89 ± 3.36
Control (R)	−3.0 ± 2.71	1.6 ± 2.43	2.19 ± 2.88	0.78 ± 2.48
Bt (S)	−4.9 ± 1.86	−0.09 ± 2.51	2.22 ± 2.52	1.07 ± 1.75
Bt (R)	−4.1 ± 3.12	2.45 ± 1.55	4.26 ± 1.11	3.69 ± 2.08

Weights were compared using one-way ANOVA. N = 20 Bt(R), Control (S) and (R); n = 22 Cry1Ac (S)

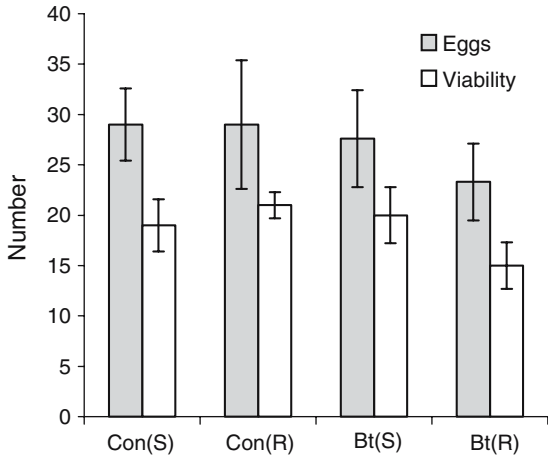

Fig. 3 Effects of Bt Cry1Ac toxin produced in transgenic canola on fecundity and egg viability of *P. madidus* over a 28-day period when fed Bt(S) or Bt(R) prey from control canola and Bt(R) or Bt(S) prey fed Cry1Ac canola. Number of eggs laid and viability were compared using one-way ANOVA. Points and bars represent mean values ± standard error. (n = 10 Bt (R), Control; n = 11 Cry1Ac (S))

Different feeding behaviour patterns were observed for adult female and male *P. madidus*, with prey selection (choice of either Bt (S or R) or control prey) being different between the sexes (Fig. 4). Both starved and pre-fed females showed a significant selection preference for the control prey type (Mann–Whitney U-test; starved $p = 0.004$, pre-fed $p = 0.0003$) over the Bt(S) prey type and similarly both starved and pre-fed females showed a significant preference for Bt(R) prey type over the Bt(S) prey type (Mann–Whitney U-test; starved $p \leq 0.0001$, pre-fed $p = 0.0003$). Male beetles, on the other hand, showed no significant selection preference for any prey type, selecting the Bt(S) prey as frequently as control or Bt(R) prey. However, despite the preference exhibited by females, there was no significant difference in the total number of ''interactions'' made by either female or male beetles with the different prey types (Fig. 5). Analysis of mean prey handling times showed that female beetles (both starved and pre-fed) spent significantly (Mann–Whitney U-test (susceptible starved $p = 0.002$, pre-fed $p = 0.0005$, resistant starved $p = 0.0002$, pre-fed $p \leq 0.0001$)) longer (Fig. 6) feeding on control prey or Bt(R) prey than Bt(S) prey. Again no differences in male beetle prey handling times

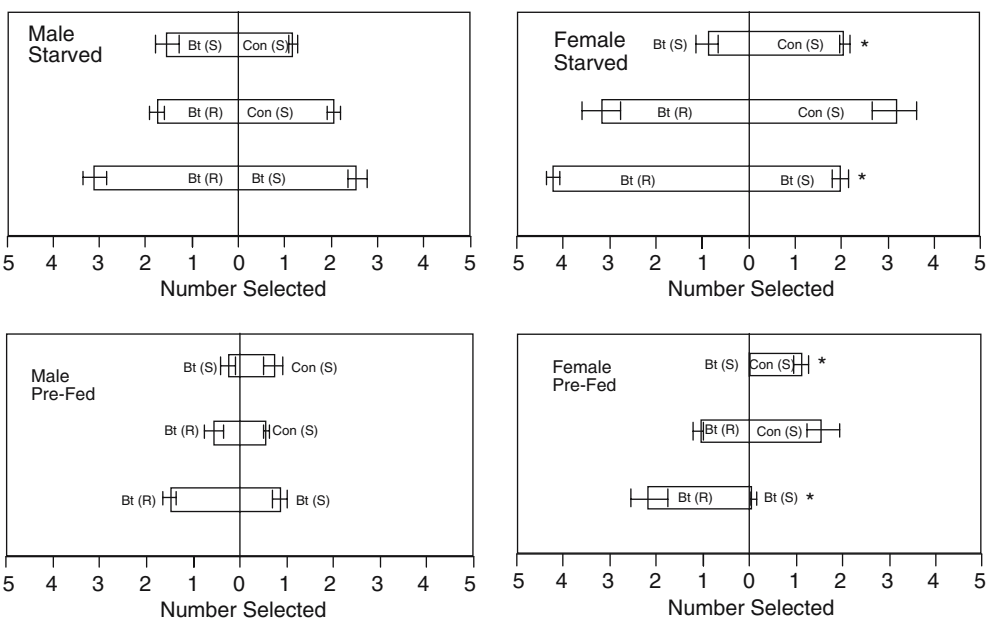

Fig. 4 Prey choice made by male and female *P. madidus* adults. Prey types: Bt(R) vs. Bt(S); Bt(R) vs. Con(S); Bt(S) vs. Con(S); beetles were either starved or pre-fed with *Calliphora* sp. prior to introduction into trials. Comparisons were made using the Mann–Whitney U-test. (n = 16 male, 16 female). * indicates significant difference *$p \leq 0.05$

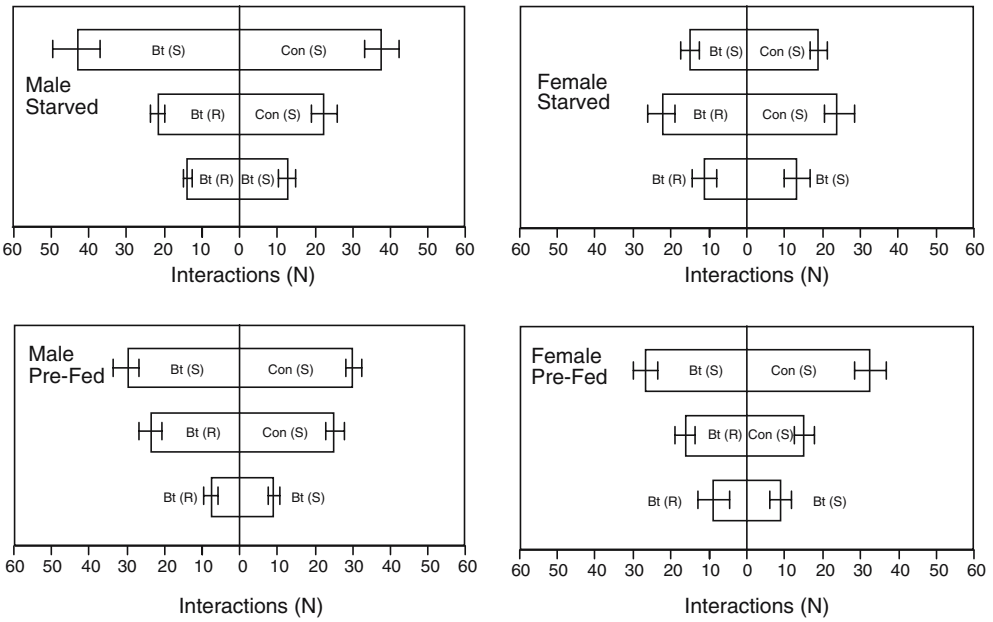

Fig. 5 Mean number of interactions between predator and pest/prey. Number of contacts made by male and female *P. madidus* with each prey type: Bt(R) vs. Bt(S); Bt(R) vs. Con(S); Bt(S) vs. Con(S); beetles were either starved or pre-fed with *Calliphora* sp. prior to introduction into trials. Comparisons were made using the Mann–Whitney *U*-test (n = 16 male, 16 female)

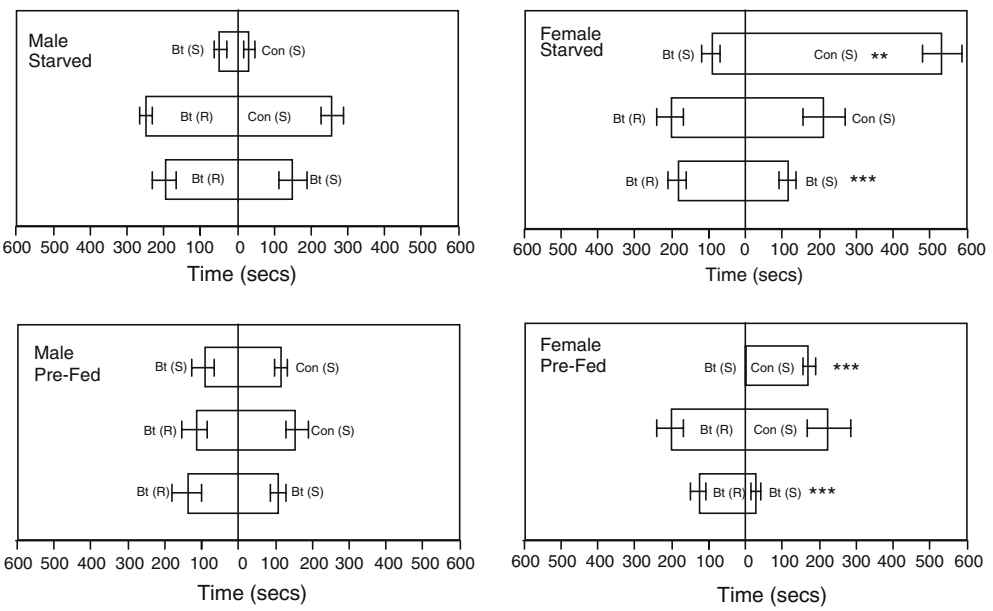

Fig. 6 Mean time spent by either male or female *P. madidus* adults handling (in contact with) prey items: Bt(R) vs. Bt(S); Bt(R) vs. Con(S); Bt(S) vs. Con(S). Comparisons were made using the Mann–Whitney *U*-test.). (n = 16 male, 16 female). * indicates significant difference (*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$)

were found. Furthermore, while there was no significant difference in the weight of Bt(S), Bt(R) or control prey consumed by male beetles, female beetles consumed significantly more control prey (Students t-test; $p = 0.03$) or Bt(R) prey (Students t-test; $p \leq 0.0001$) compared to

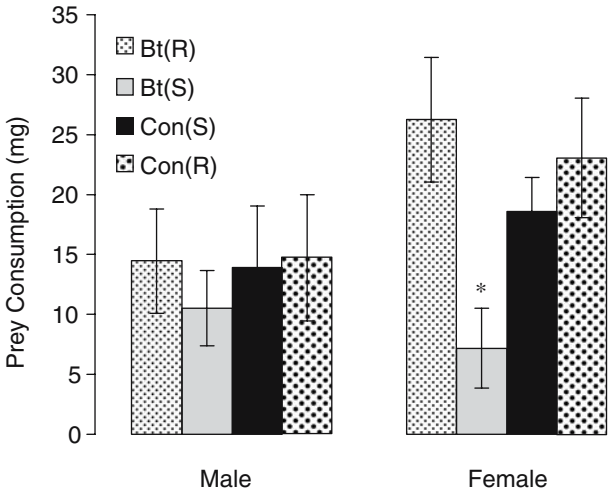

Fig. 7 Mean daily prey consumption by both female and male *P. madidus* when fed Bt(R), Bt(S) or Con(S) prey types. Comparisons were made using the Student's *t*-test. (n = 16 male, 16 female). * indicates significant difference *$p \leq 0.05$

Bt(S) prey (on the rare occasions when it was selected Fig. 7).

No significant differences in any of the parameters tested (number of interactions, prey choice, prey handling time or daily prey consumption) for male or female beetles, whether starved or pre-fed, were observed in choice tests between control(S) prey versus Bt(R) prey (Figs. 4–7).

Preliminary studies showed no preference for Con (S) vs Con (R) thus only Con (S) was used as a control due to availability.

Discussion

The only insect-resistant transgenic crops to have been commercialised are those expressing Bt Cry toxins (James, 2005). Following their commercialisation, many concerns have been expressed regarding potential impact on the environment, not least on their potential impact, on non-target organisms. Whilst most field studies to date have shown minimal impacts on non-target beneficial organisms, some laboratory studies have shown negative effects (Ferry et al. 2003a).

Transgenic canola plants used in the present study were shown to produce Cry1Ac at levels of up to 0.06% total soluble protein; these values are within the ranges reported for expression of other *cry* genes (Stewart et al. 1996). Immunoassay of *P. xylostella* larvae clearly demonstrated the presence of Cry1Ac in both Bt (S) and Bt (R) prey, thus confirming that predatory carabids, which consume the whole larvae including the gut, would become exposed to the protein. This result is consistent with other recent studies (Head et al. 2001; Dutton et al. 2002; Ferry et al. 2003b) that not only demonstrate the risk of secondary exposure via predation of phytophagous pests, but additionally demonstrate biological activity following ingestion.

Although these plants significantly reduced survival and development of the pest *P. xylostella*, thus confirming previous studies (Ramachandran et al. 1998), no short-term deleterious effects on the carabid, when mediated by the prey, were observed, even when exposed to higher levels of Bt following consumption of Bt (R) *P. xylostella*. These results are perhaps contrary to what might have been predicted since some carabids have been shown to be sensitive to changes in prey quality over relatively short time periods. For example, Meissle et al. (2005) demonstrated that Bt-dosed prey deleteriously affected carabids; however, it was not clear whether effects were direct (toxicity of Bt) or indirect. The results presented here strongly suggest that Bt Cry1Ac toxin has no direct impact on *P. madidus* adults as evidenced by 100% survival on Bt (R) prey. However, a trend towards lower survival in the Bt (S) group suggests negative effects resulting form poor prey quality.

Whilst larvae may be more sensitive to pesticides, and sub-optimal food sources, the present

study focused on adults, since not only is it possible to measure the effects of acute toxicity on parameters such as survival and body mass change (Unal and Jepson 1991), but it is also possible to measure more subtle effects such as that of fecundity, which are likely to be more important ecological parameters compared to their effects on short-term survival. Furthermore, adult carabids are more likely to be exposed to the Bt toxin since the larvae of *P. madidus* spend much of their time underground and consume different prey types (Luff, 1974). Previous studies have shown that there is a strong relationship between the quality/quantity of food ingested and subsequent fecundity in adult carabids, including *P. madidus* (van Dijk, 1994; Wallin et al. 1992; Ferry et al. 2005). Results from the present trials, however, showed that exposure to Bt Cry1Ac toxin did not significantly affect the fecundity of the carabid, with eggs from all groups being equally viable. This result is consistent with studies on female ladybeetles (*H. axyridis*) where consumption of another insecticidal protein, oryzacystatin-1 (OC-1), did not have any significant impact on either fecundity or subsequent egg viability following prolonged exposure to the protease inhibitor (Ferry et al. 2003b).

In contrast to work reported here, Hilbeck et al. (1999) and Dutton et al. (2002, 2003b) showed that consumption of Bt-poisoned prey had a negative impact on the green lacewing, *Chrysoperla carnea* and that the biopesticide Dipel® had negative effects on survival, development and weight gain (Dutton et al. 2003b). Based on their results, they suggested that the sub-lethal effects of Dipel® on the prey (*Spodoptera littoralis*) resulted in altered amino acid composition in the prey, which in turn limited essential amino acids required by the lacewing larvae. Carabid beetles are highly polyphagous predators and are known to scavenge prey, and as such, may be less susceptible to the effects of low prey quality than lacewings. Furthermore, there was no evidence from the bioassay to suggest that carabids compensated for poor prey quality by increased prey consumption. Male beetles showed no significant differences in consumption of prey from any of the groups tested, whilst female beetles actually consumed significantly less Bt (S) prey than other treatments, yet suffered no significant effects on reproductive fitness. Both female and male *P. madidus* killed and consumed a single prey type in 'no-choice' experiments. In 'free-choice' trials, both control and Bt (R) prey were selected in preference to Bt (S) prey. However, no difference in 'interactions' with prey type was found, suggesting that although female *P. madidus* contact Bt (S) prey items as frequently as control or resistant prey, they choose to avoid it; even when starved, Bt (S) prey was not acceptable. Prey handling time showed that female beetles (both starved and prefed) spent significantly less time feeding on Bt (S) prey, suggesting that on the occasion when it was selected, it was only partially consumed and then rejected. Female beetles were also seen to consume a significantly smaller weight of Bt (S) prey, such differences in female and male *P. madidus* feeding behaviour has previously been reported (Mair and Port 2001). This discrimination ability could enable generalist predators to cope with high variability in the quality of the numerous prey types encountered (Roger et al. 2001). The fact that *P. madidus* avoid Bt (S) prey in a choice situation suggests that control or Bt (R) prey are selected as they are of higher quality; however, Bt (S) prey provide sufficient nutrition to support reproduction when beetles are confined to this single prey type in bioassay. Invertebrate predators often face periods of prey scarcity, and in the absence of alternative prey they have been shown to attack and consume inferior prey (Eubanks and Denno 2000).

Meissle et al. (2005) similarly conducted paired choice tests on *Poecilus (Pterostichus) cupreus* and conversely found no avoidance of Bt vs. non-Bt lepidopteran larvae; this highlights the importance of considering individual species behaviour when formulating risk assessment strategies. Whilst both *P. cupreus* and *P. madidus* are members of the *Pterostichini*, these two species have different feeding habits. *P. madidus* has been shown to have subtle interactions in terms of prey choice with sex and stage of sexual maturity affecting prey selection (Mair and Port 2001). Furthermore, *P. xylostella* are susceptible to Bt toxins, whereas *S. littoralis* used in the previous study were partially tolerant. This would result in

prey being in a better condition than *P. xylostella* and may explain avoidance in this present study but not with *P. cupreus*.

When developing risk assessment strategies, it is important to place the organism in an ecological context. Since carabid beetles are generalist predators, with lepidopteran larvae normally making up only a small proportion of their diet (Lovei and Suderland 1996), it is highly unlikely that carabids would be unable to find alternative prey. This situation also applies to the green lacewing, the only beneficial insect for which a negative effect of Bt-plants has been reported. Prey quality effects resulted in higher mortality when *C. carnea* was fed Bt Cry1Ab poisoned *Spodoptera littoralis* larvae than when fed other pest species, which had similarly consumed Bt expressing plants. Since *C. carnea* are known to preferentially consume aphids rather than lepidopteran larvae (Meier and Hilbeck 2001), they would rarely be confined to a diet of lepidopteran larvae in the field; thus prey choice and predator behaviour may mitigate negative effects resulting from poor prey quality.

In the present study, *P. xylostella* was used as a model pest to assess the effects of prey-mediated exposure to insecticidal proteins expressed in transgenic plants. This study thus enabled the potential effects of deploying insect-resistant transgenic crops on beneficial predators to be evaluated in a meaningful way. Despite the potential for both direct (from Bt itself) and prey-mediated effects, expression of Bt cry1Ac in transgenic oilseed rape plants did not cause any overall significant effects on survival, body mass change, fecundity, or egg viability. However, the results also demonstrated that females of this species of carabid avoid consumption of 'sick' prey in the presence of alternative prey. Thus the potential impact of Bt toxin Cry1Ac on adult carabids is minimal, both in terms of direct toxicity and of poor prey quality. Furthermore, the impact of poor prey quality may be mitigated by behavioural avoidance of sub-optimal prey. In conclusion, the cultivation of Bt canola crops to control lepidopteran pests would have a limited direct impact on carabid population processes. This study further highlights the difficulty of selecting a single test species in risk-assessment studies. The behaviour of *P. madidus* is shown to be significantly different from the carabid previously suggested as general test organism (Dutton et al. 2003a).

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