Absence of non-target effects of two *Bacillus thuringiensis* coleopteran active δ-endotoxins on the bulb mite, *Rhizoglypus robini* (Claparède) (Acari, Acaridae)

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Abstract: Transgenic crops with plant-incorporated protectants are often more specific than synthetic insecticides and have the potential to reduce impacts on non-target organisms. In this study we assessed the impact of Cry3Aa and Cry3Bb1 coleopteran-active δ-endotoxins on the bulb mite, *Rhizoglypus robini*. The effect of Cry3Aa prototoxin in solutions of the biopesticide Novodor® on mite survival was assessed in laboratory studies. Survival of *R. robini* exposed to Cry3Aa in a short-term contact and ingestion experiment was not affected, although *R. robini* was significantly affected by the insecticide Fipronil® used as a positive control. Similarly, *R. robini* exposed in a longer duration feeding trial to the Cry3Aa toxin in artificial diet were also not significantly affected. When Cry3Aa was tested on the positive control insect, *Leptinotarsa decemlineata*, reduced weight of larvae and increased mortality was recorded. The effect of Cry3Bb1 toxin in transgenic corn tissues on *R. robini* food choice was assessed in a laboratory study. In no-choice tests a greater proportion of *R. robini* were found on garlic roots than on Cry3Bb1 transgenic corn and a near-isogenic non-transgenic corn. In a choice test, more *R. robini* was recovered on garlic roots than on either corn variety, and on Cry3Bb1 corn than on non-transgenic corn. In large field plots using specific mite traps across the growing season, *R. robini* mite populations were not significantly different between Cry3Bb1 corn and non-transgenic corn alone or non-transgenic corn treated with different combinations of two insecticides. Our results, combined with results from other studies, suggest that transgenic plants expressing the Cry3Aa or Cry3Bb1 *Bacillus thuringiensis* toxins are likely to have negligible impact on *R. robini* mite populations.

Key words: *Rhizoglypus robini*, Acaridae, bulb mite, non-target effects, transgenic plants

1 Introduction

Since 1995 crop plants have been genetically altered to incorporate genes that code for synthesis of naturally occurring, insecticidal *Bacillus thuringiensis* (Bt) toxic proteins in plant tissues (Ostlie et al., 1997). Globally, 7.8 million hectares were planted to Bt crops in 2001 (James, 2001). Bt toxins are much more specific than broad-spectrum synthetic insecticides, with activity usually restricted to one insect order. They have a very low mammalian toxicity, and cannot drift or runoff to non-target areas, a common problem of large-scale application of chemical insecticides (Betz et al., 2000; Siegel, 2001).

Although the majority of acreage planted to Bt crops is for control of above ground pests of corn, cotton, and potato (US EPA, 2001) the plants can also express these toxins in below-ground tissues. The direct control of root-feeding insects in production systems is hampered by difficulties in detection, access to the target pest and safety of the most commonly applied chemical products (Hunter, 2001). New transgenic corn varieties targeted against below-ground pests of corn are now in development (Ostlie, 2001) and in registration review by the EPA (Environmental Protection Agency) (Ag Biotech Info Net, 2002a). This new transgenic corn is targeted to control immature stages of the western corn rootworm, *Diabrotica virgifera virgifera* (LeConte) (Col., Chrysomelidae) whose emerging larvae feed on the developing roots of young corn plants (Pears and Pilcher, 2002). Rotation of corn with another crop, primarily soya bean, has been the primary pest management tactic for rootworm control (Univ. of Illinois, 2002). A new strain of adult rootworms that will lay their eggs in soya bean fields makes crop rotation less effective as a management tool (Moellenbeck et al., 2001; Levine et al., 2002), leading to an increase in insecticide use (Univ. of Illinois, 2002). Non-target insects and mites are key components of the agricultural landscape (Amano and Haseeb, 2001).
The effect of application of new insecticidal technologies on the biodiversity of non-target organisms should be assessed (Poppy, 2000; Hilbeck, 2001; Schmidt and Hilbeck, 2001; AG BIOTECH INFO NET, 2002b). For example, although no catastrophic non-target impacts of transgenic corn have yet been identified, studies have yielded varying results (Obrycki et al., 2001). Non-target effects on mite species are not required for EPA registration of transgenic plants, and only a small number of studies have been carried out (Chapman and Hoy, 1991; Yu et al., 1997; Lozza et al., 2000).

In this study we tested for effects of two Bt δ-endotoxins found in underground tissues of transgenic plants on a common non-target organism, the bulb mite, Rhizoglypus robini (Claprède). Bulb mites are primarily associated with plants in the family Liliaceae but are also known to affect other agricultural crops including potatoes (Diaz, 1998; Diaz et al., 2000). Rhizoglypus robini is best classified as a generalist, as it will thrive on most natural foods it is offered including plant parts, manure, macerated soil arthropods (Gerson et al., 1985) and fungi (Woody and Fashing, 1993).

The two toxins tested, Cry3Aa and Cry3Bb1, are δ-endotoxins active against Coleoptera in the family Chrysomelidae (Donovan et al., 1992). Cry3Aa is isolated from B. t. ssp. tenebrionis. It is engineered as a modified gene encoding a full-length protein identical in amino acid sequence to the protein produced by B. t. ssp. tenebrionis, into Bt potatoes to control the leaf-feeding Colorado potato beetle, Leptinotarsa decemlineata (Say) (Col., Chrysomelidae) (Perlak et al., 1993). The toxic protein is also found in tubers (US EPA, 2001). The protoxin containing the active Cry3Aa protein crystal is incorporated into Novodor® and is EPA registered as a biopesticide for control of L. decemlineata and the elm leaf beetle [Xanthogaleruca luteola (Muller)] (Col., Chrysomelidae) (Agrobiologicals, 2002). A recent study showed no significant effects of a Cry3Aa-based microbial spray or transgenic Bt potatoes on the abundance of above-ground non-target beneficial predators and secondary pests in potato fields (Reed et al., 2001). The second toxin tested is a version of the Cry3Bb1 toxin isolated from B. t. ssp. kumamotoensis that is active against L. decemlineata and the corn rootworm (Diabrotica) complex (Donovan et al., 1992). The toxin has been modified to increase its activity against corn rootworm species and the Monsanto Corporation has applied to the EPA for registration approval of a transgenic corn (MON 863) with the gene to produce this modified Cry3Bb1 toxin (Ditto, 2002).

The short and long duration contact and ingestion experiments were developed to have a wide applicability to test the effects of either Bt expressing plant tissues or Bt toxins (Sms, 2000). Additionally, we surveyed mites in fields where they could be exposed to Cry3Bb1 transgenic corn plant tissue directly or to the toxin in corn plant exudates in the soil (Saxena et al., 2002) over the growing season.

2 Materials and Methods

2.1 Rhizoglypus robini colony

Rhizoglypus robini mites were obtained from a colony maintained at the New York State Agricultural Experiment Station Hudson Valley Laboratory of Cornell University. The mite colony was cultured on a pinto bean based artificial diet (Shorey and Hale, 1965) in 59.2 ml disposable plastic cups. Ten-gram low melting point agar was mixed with 230 ml water and boiled for 10 min. The agar was cooled to below 70°C and subsequently blended with 160 g pre-soaked pinto beans, 24 g brewers yeast, 2.5 g ascorbic acid, 1.5 g methyl paraben, 1.0 g sorbic acid, 2.5 ml 37% formaldehyde (aqueous) and 250 ml distilled water. The cups of mites were kept in a growth chamber in the dark at 22°C, RH approximately 75%, in tightly closed wooden boxes. The colony was maintained by transferring a subset of mites to new diet cups approximately every 4 weeks. Mites were staged and sexed by the presence and placement of genitalia (Masson, 1972). In short-duration toxicity tests and plant tissue tests mites were food-deprived for 24 h by removing pairs of adult female and male mites in copula from the artificial diet and placing them in a Falcon® 50-mm sealing Petri dish filled with 4 ml of water. This technique kept the mites from dehydrating, without affecting their survival up to 5 days (Gerson et al., 1983). Mites in water were kept in a growth chamber at 22°C and RH 75% in total darkness prior to setting up an experiment.

2.2 Toxin concentrations

For short- and long-duration toxicity tests of Cry3Aa, the amount of Novodor® (Abbott Laboratories, EPA Reg. No. 275-118, North Chicago, IL, USA) used for the amount of active ingredient (AI) tested was calculated from the proportion of AI given on the product label and the weight-to-volume ratio of the product. The Novodor® we tested had 3% Bt. tenebrionis active toxin (AI) per volume. The Fipronil® (Rhône-Poulenc Ag. Co., EPA Est. No. 264-NC-1, Lyon, France) we tested had 3% AI per dry weight of the wettable powder formulation.

Mon 863 Cry3Bb1 corn has an average of 100 p.p.m. of toxin in the root tissue (T. Vaughn, pers. comm.).

2.3 Short-duration contact and ingestion exposure of R. robini to Cry3Aa toxin

To assess the combined effects of short-term contact and ingestion toxicity we used the Cry3Aa containing biopesticide Novodor®. We adapted methods similar to ones used for efficacy testing of chemical pesticides against bulb mites (Chen, 1990; Zhang et al., 1996). A bioassay container was constructed from a 2.5-cm scintillation vial into which was inserted a 2-cm disc of black construction paper. This paper was sturdy enough to withstand wetting for the duration of the tests and was visible in the gut of each mite after consumption (Gerson et al., 1983).

Pairs of adult female and male mites were removed from the soaking dishes and exposed to one of seven different treatments: four concentrations of Cry3Aa in Novodor®, two concentrations of the positive control, a phenyl pyrazole neurotoxin in the insecticide Fipronil®, and the negative control, distilled water. There were five pairs (10 mites) per vial and each treatment was replicated five times (50 mites total for each treatment). Fipronil® interferes with normal neural function and acts on insects and mites by both...
ingestion and contact (National Pesticide Telecommunications Network, 1997). Solutions of Novodor® and Fipronil® were prepared in distilled water and 0.3 ml of a solution was measured into a bioassay container. A total of 10 mites (five pairs) were placed in one treatment vial. Capped vials were kept in a wooden box inside a growth chamber at a temperature of 22°C and RH 75%. Tests ran for 48 h, at which time mites were removed from each vial and scored. In preliminary tests the Fipronil® treatment did not kill the mites but it clearly affected their locomotor abilities. Therefore, mites were scored in the following categories: healthy, dead, alive but unable to move forward one body length when stimulated, (healthy mites moved readily), or missing. Proportional data was arcsine transformed before analysis with an ANOVA to look for a treatment effect and concentration means were compared with a Bonferroni t-test (PROC GLM, SAS Institute, 1990).

2.4 Feeding trials of *R. robinii* and *L. decemlineata* on artificial diet containing Cry3Aa toxin in Novodor® solution

Artificial diet for *R. robinii* mites was prepared and changed weekly. A small amount of green food colouring was added to facilitate location of the mites in the observation cages. Two batches of diet were prepared: one batch was the control treatment and the other batch incorporated 100 p.p.m. of Cry3Aa in Novodor® solution and was the experimental treatment. Novodor® solution was added last to the cooled diet. The mites were reared in modified Plexiglass observation cages (Gerson et al., 1983). The cage base was 7.5 cm long, 2.5 cm wide and 2 mm thick, with three evenly spaced, 12-mm holes drilled through the base. A piece of black construction paper the same size as the base was placed on top, misted lightly with water, and a thumb depression was made over each hole. To this depression was added a small spatula of either control or treatment diet and one *R. robinii* mite. A glass microscope slide was scored twice and broken into three equal-sized rectangles, one to cover each observation cage. The rectangles of glass, construction paper and base were held together with two small document clips. A set of three observation cages all contained either treatment or control diet. All cages were stored in sealed plastic containers with a moist paper towel on the bottom. The experiment was carried out in darkness in a growth chamber at 22°C and RH 85%. Artificial diet was changed weekly at which time each mite was counted. Mites that were missing were assumed dead, as mites were unable to escape from the observation cages. Artificial diet was prepared on four different dates. The duration of the experiment was 3 weeks. There were 30 adult female and 30 adult males mites in each treatment (60 per treatment, 120 total). At the end of the 3-week test, mites were scored as alive (normal) or dead (included missing). Differences in survival among the four groups was tested with a Fisher’s exact test for 2 x 4 independent samples and between the treatment and control group, and between males and females combined from both treatments, with a Fisher's exact test for 2 x 2 independent samples (PROC FREQ, SAS Institute, 1990).

To assess whether or not the Cry3Aa in Novodor® would be effective in this type of experimental design, a preliminary experiment was carried out with one of the target insects for this biopesticide, *L. decemlineata*. *Leptinotarsa decemlineata* eggs were purchased from the Philip Alami Beneficial Insect Lab, Trenton, NJ. Eggs were kept in Petri dishes on moist Kimwipes in a growth chamber at 22°C, L/D cycle 16 : 8, 85% RH until hatching. First instar larvae were transferred to cut non-transgenic potato foliage from greenhouse-grown culture. The potato stems were placed in water-filled aquapics and larvae were reared in paper towel-lined plastic boxes kept in the growth chamber. When larvae moulted to the second instar (first instar larvae are reluctant to feed on artificial diet) they were transferred to artificial diet cups. Artificial diet specific for *L. decemlineata* (Sims, 2000) was prepared in five separate batches. A batch of diet consisted of 3.25 g low melting point agar added to 116 ml water brought to a boil and poured into a blender. Cold water (58 ml) was added followed by 37 g *L. decemlineata* diet (BIOSERV® #F9380B), 2.5 g commercial potato flakes, 0.25 g methyl paraben, 0.25 g sorbic acid and 0.75 ml formaldehyde (37%). One batch was control diet. The other batches contained increasing concentrations of Cry3Aa in Novodor® solution, added as the last ingredient to the cooled diet. Diet was poured into 29.6-ml disposable plastic cups with lids. For each treatment and the control, there were 51–53 diet cups per treatment. One second instar *L. decemlineata* was added to each diet cup. Cups were stored in a growth chamber at the above conditions. After 10 days larvae were recorded as alive or dead, and surviving larvae were weighed. The effect of treatment on the weight of surviving larvae and effect of treatment concentration were compared with an ANOVA and a Bonferroni t-test (PROC GLM, SAS Institute, 1990).

2.5 Choice and no-choice attraction of garlic, Cry3Bb1 corn roots and non-transgenic corn roots to *R. robinii*

To assess the relative attractiveness of Cry3Bb1 corn roots, non-transgenic corn roots and garlic (a preferred host used as a positive control) to *R. robinii*, we recorded the proportion of mites recovered from the various treatments, alone and in combinations. The corn seeds planted were Bt Cry3Bb1 corn hybrid seed treated with Gauch® insecticide (BT-G) and Captan® fungicide and a near-isogenic non-transgenic corn seed treated with Gauch® insecticide (NONBT-G) and Fludioxonil® and Metalaxyl® fungicides. Corn seeds were germinated in course vermiculite in 13.5-cm tubes in racks in a greenhouse and allowed to grow for 3 days. At this time the corn seedlings were at the two-leaf stage. Prior to testing, vermiculite was poured from the tubes and the corn roots were cut from the plant below the seed and rinsed twice with deionized water to remove any residual vermiculite. Commercial garlic cloves were peeled and cut in approximately 2 cm long x 2 mm wide strips for testing. Plant material was kept moist between sheets of dampened paper towels. Mites were tested in 40-mm Petri dishes that had a circle of white Whatman #1 filter paper cut to fit the bottom. The filter paper was misted with deionized water before the plant material or mites were added. Ten adult mites (five pairs from a soaking dish) were placed in the middle of the filter paper at the start of a test.

In no-choice tests, only one of the three treatments, Bt corn roots (BT-G), non-transgenic corn roots (NONBT-G), or garlic, was added to a dish. Four pieces of corn root or garlic were placed in a diamond-shape along the inside periphery of the dish. Covered experimental dishes were put on a flat tray, staggered by treatment, and the tray was kept in a 25°C growth chamber, in the dark, for 24 h. For each of the three no-choice treatments there were 30 tests, for a total of 90 no-choice tests. A trial had 10 tests of each treatment, and there were three trials in total. At 24 h the number of mites found on the corn roots or garlic was counted. Proportional data was arcsine transformed before analysis with an ANOVA to look for trial and treatment effects. The mean numbers of mites recovered were compared with a Bonferroni t-test (PROC GLM, SAS Institute, 1990). In the analysis there was no significant effect (P ≥ 0.42) of trial,
therefore this parameter was removed from the model and tests from all trials were pooled. In choice tests the procedure was identical except for the following: one side of an experimental dish contained two pieces of one treatment while the other side had two pieces of a different treatment. There were three trials of 30 choice tests (90 tests total). In each trial, there were 10 tests each of the following choices: BT-G corn roots vs. garlic, NONBT-G corn roots vs. garlic, and BT-G corn roots vs. NONBT-G corn roots. For each comparison, the number of mites found was tested with a Wilcoxon two-sample test with continuity correction of 0.5 (PROC NPARIWAY, SAS Institute, 1990).

2.6 Field survey of *R. robini* in transgenic and non-transgenic corn plots

Naturally occurring populations of *R. robini* were surveyed in five treatments of field corn on five occasions from 12 June to 28 August 2001 in Freeville, NY. One corn treatment was four plots of Cry3Bb1 corn hybrid seed treated with Gaucho® insecticide (BT-G). There were four different treatments of a near-isogenic non-transgenic seed, three plots were corn seed treated with Gaucho® insecticide (NONBT-G), four plots were corn seed without Gaucho® insecticide (NONBT-I), and three plots were corn seed without Gaucho® insecticide but planted in a plot treated with Force 3G® (tefluthrin - Zeneca Agrichemicals) soil insecticide, commonly used to control corn rootworm larvae (NONBT-I). In total there were 18 plots. Transgenic corn seed was treated with the fungicide Captan®, while all non-transgenic corn seed was treated with the fungicides Fludioxonil® and Metalaxyl®. Each corn plot (46 m long, 50 m wide) was planted by standard agronomic practices in 68 rows separated by 30".

We modified a bulb mite trap (Grasso et al., 1985) to assess *R. robini* populations in the corn plots. A 12 cm long by 3.5 cm wide piece of PVC pipe was sunk into the ground to a depth of 11 cm, leaving a 1-cm segment above the soil. This trap stand supported the trap in the soil. The trap was a 13 cm long by 2 cm wide segment of PVC pipe. A circle of window screen was glued to the underground end of the trap. Commercial, peeled, garlic cloves were used as bait and placed inside the trap on the screen. The opposite end of the trap was covered with the bottom end of a polypropylene vial with six small perforations, as a rain cap. Each trap was placed up right in the trap stand. Traps were placed two per plot, one 20-corn rows in from the left side of the plot and one 20-corn rows in from the right side of the plot (with approximately 28 rows of corn between them), both traps were 23 m from the front edge (the middle of the plot, approximately). One week later, traps were collected and the contents placed into alcohol. *Rhizoglypus robini* in alcohol were separated into adults and juveniles and counted. For both adult and juvenile *R. robini*, the number of mites trapped was compared between treatments and across dates using a repeated measures analysis (PROC MIXED, SAS Institute, 1990).

3 Results

3.1 Short-term contact and ingestion toxicity of Cry3Aa toxin and the Fipronil® control

There was no significant difference (P = 0.05) between the deionized water control and any of the concentrations of Cry3Aa in Novodor® solution as measured in survival of adult *R. robini* after 48 h (table 1). The positive control neurotoxin in Fipronil® did have a significant negative effect (P ≤ 0.05) on *R. robini*; 86–88% of adult mites were unable to move one body length after stimulation. The result from the positive control demonstrates that a toxic effect, if present, would have been recorded.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effects1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal2</td>
</tr>
<tr>
<td>Deionized water</td>
<td>0.92 a</td>
</tr>
<tr>
<td>Novodor® Cry3Aa</td>
<td>1.00 a</td>
</tr>
<tr>
<td>100 p.p.m.</td>
<td>0.98 a</td>
</tr>
<tr>
<td>250 p.p.m.</td>
<td>0.98 a</td>
</tr>
<tr>
<td>500 p.p.m.</td>
<td>0.98 a</td>
</tr>
<tr>
<td>1000 p.p.m.</td>
<td>0.98 a</td>
</tr>
<tr>
<td>Fipronil® neurotoxin</td>
<td></td>
</tr>
<tr>
<td>250 p.p.m.</td>
<td>0.08 h</td>
</tr>
<tr>
<td>500 p.p.m.</td>
<td>0.00 h</td>
</tr>
</tbody>
</table>

1 *R. robini* mites were scored as normal, dead, missing (not found) or unable to move forward one body length after stimulation.
2 Proportions followed by different letters are significantly different (Bonferroni α-test, P ≤ 0.05).

3.2 Feeding trials of *R. robini* and *L. decemlineata* on artificial diet containing Cry3Aa toxin in Novodor® solution

There was a significant difference (P = 0.043) in survival among the four groups of mites tested, male and female *R. robini* on control and Novodor®-treated diet. Combined results from both treatments showed a greater proportion of males survived when compared with the proportion of surviving females (P = 0.014), while combining males and females within a treatment showed no significant treatment effect (P ≥ 0.05, fig. 1a).

As expected, there was a significant effect of Cry3Aa on growth of *L. decemlineata* larvae (ANOVA, n = 230, P ≤ 0.0001, fig. 1b). There was a significant concentration effect (P ≤ 0.05). Surviving larvae gained less weight when Novodor® was present in the diet, starting at a concentration of 10 p.p.m. Mortality was increased in the 1000 p.p.m. treatment (fig. 1b).

3.3 Choice and no-choice tests of attraction of Cry3Bb1 corn roots, non-transgenic corn roots and garlic

*Rhizoglypus robini* mites not found on plant tissues were found on the filter paper. A small proportion of mites not found were recorded as missing and not entered into the analysis.

In no-choice tests, there was a significant treatment effect (P = 0.0217). A higher proportion of *R. robini* mites were recovered on garlic than on NONBT-G corn roots (P ≤ 0.05, fig. 2). The proportion of
R. robini recovered on BT-G corn roots was not significantly different from garlic or NONBT-G corn roots ($P \geq 0.05$).

In choice tests, significantly more R. robini mites were found on garlic when compared with BT-G corn roots and NONBT-G corn roots ($P \leq 0.0001$, fig. 3, expts 1 and 2). BT-G corn roots were preferred over NONBT-G corn roots ($P \leq 0.0056$) (expt 3). In general, recovery of R. robini mites on roots was high (85–93%) when garlic, a preferred

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**Fig. 1.** (a) The proportion of adult Rhizoglypus robini mites surviving after feeding for 3 weeks on control diet or Cry3Aa toxin diet. Combined results from both treatments showed a greater proportion of surviving males in comparison with the proportion of surviving females (Fisher’s exact test for $2 \times 2$ independent samples, $P = 0.014$), while combining males and females within a treatment showed no significant treatment effect (Fisher’s exact test for $2 \times 2$ independent samples, $P \geq 0.05$). (b) The mean (± SE) weight in mg of Leptinotarsa decemlineata larvae reared on artificial diet containing increasing concentrations of Cry3Aa toxin. Treatments with different letters are significantly different (Bonferroni t-test, $P \leq 0.05$). Numbers in the columns are the proportion of larvae surviving per treatment at the end of the experiment.

**Fig. 2.** The mean proportion (± SE) of Rhizoglypus robini mites found on plant tissues in a no-choice experiment of garlic bulb slices, BT-G or NONBT-G corn roots. For each treatment there were 10 tests each testing 10 mites, replicated three times for a total of 30 tests per treatment, 90 tests total in the experiment. Treatments with different letters are significantly different (Bonferroni t-test, $P \leq 0.05$).

**Fig. 3.** The mean proportion (± SE) of Rhizoglypus robini mites found on plant tissue in three different choice experiments. For each experiment there was two tissue types tested in 10 trials each testing 10 adult mites, replicated three times, for a total of 30 tests per experiment. Treatments with different letters are significantly different (Wilcoxon two-sample test with continuity correction of 0.5; expt 1, $Z = -7.14$, $P \leq 0.0001$; expt 2, $Z = -7.20$, $P \leq 0.0001$; expt 3, $Z = -2.77$, $P \leq 0.0056$) preferred over NONBT-G corn roots ($P \leq 0.0056$) (expt 3). In general, recovery of R. robini mites on roots was high (85–93%) when garlic, a preferred
Table 2. Number of adult and immature Rhizoglypus robini mites caught in the soil in garlic-baited traps from plots of five different treatments of field corn in Freeville, NY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of traps</th>
<th>Adult mites&lt;sup&gt;1,4&lt;/sup&gt;</th>
<th>Immature mites&lt;sup&gt;1,4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT-G</td>
<td>40</td>
<td>2.55 ± 0.70 a</td>
<td>17.68 ± 7.27 a</td>
</tr>
<tr>
<td>NONBT-G</td>
<td>30</td>
<td>1.33 ± 0.37 a</td>
<td>16.93 ± 5.24 a</td>
</tr>
<tr>
<td>NONBT</td>
<td>30</td>
<td>3.28 ± 0.89 a</td>
<td>25.85 ± 9.86 a</td>
</tr>
<tr>
<td>NONBT-I</td>
<td>40</td>
<td>3.85 ± 2.02 a</td>
<td>18.70 ± 8.16 a</td>
</tr>
<tr>
<td>NONBT-G-I</td>
<td>30</td>
<td>3.50 ± 2.01 a</td>
<td>16.33 ± 5.01 a</td>
</tr>
</tbody>
</table>

<sup>1</sup> The corn treatments were BtCry3Bb1 corn hybrid seed treated with Gaucho<sup>®</sup> insecticide (BT-G), a near-isogenic non-transgenic seed treated with Gaucho<sup>®</sup> insecticide (NONBT-G), a near-isogenic non-transgenic seed without Gaucho<sup>®</sup> insecticide (NONBT), a near-isogenic non-transgenic seed without Gaucho<sup>®</sup> insecticide (NONBT-I), and a near-isogenic seed treated with Gaucho<sup>®</sup> insecticide and the plot treated with Force 3G<sup>®</sup> insecticide (NONBT-G-I).

<sup>2</sup> Two traps per plot sampled five times multiplied by the number of replicate plots (three or four).

<sup>3</sup> There was no significant difference among treatments in the mean numbers of adult mites collected over the five sampling dates (repeated measures analysis, P = 0.82).

<sup>4</sup> Numbers followed by the same letter are not significantly different (Bonferroni i-test, P ≥ 0.05).

<sup>5</sup> There was no significant difference among treatments in the mean numbers of immature mites collected over the five sampling dates (repeated measures analysis, P = 0.89).

In general, there is high specificity of receptor-binding sites for the Bt δ-endotoxins, which is responsible for their lack of action against non-target organisms (Betz et al., 2000). Reproduction of the oribatid mite, Oppia nitens (Koch), was unaffected when fed cotton leaves containing the δ-endotoxins Cry1Ab and Cry1Ac (Yu et al., 1997). No mite-specific bacteria have been isolated (Poinar and Poinar, 1998).

4 Discussion

In the short-term contact and ingestion experiment there was no evidence of a toxic effect of Cry3Aa toxin on R. robini. The longer-duration feeding trials did not show an increase in mortality when R. robini mites were exposed to the biopesticide Novodor<sup>®</sup> containing the Cry3Aa toxin within the range of concentrations that did have sublethal and lethal effects on L. decemlineata. In a study by Chapman and Hoy (1991) preparations of B.t. ssp. tenebrionis was moderately toxic to immature stages of the predatory mite Metaseiulus occidentalis (Nesbitt) but not to the two-spotted spider mite, Tetranychus urticae (Koch). However, the authors were hesitant to speculate on the cause of this effect or to predict whether field studies would complement the laboratory results.

In general, in both choice and no-choice tests, R. robini mites were found on their preferred host, garlic, more frequently than on corn roots. Rhizoglypus robini mites were found more often on BT-G corn roots containing the toxin Cry3Bb1 in comparison with NONBT-G corn roots. One possible explanation for this effect is slight differences in chemical or physical properties of the near, but not identical, isogenic lines of corn tested (G. Head, pers comm.). A second possible explanation is a repellent effect of the fungicides used as seed treatments on the NONBT-G seeds, in comparison with the fungicide on BT-G corn seeds. Although the corn roots were rinsed twice with distilled water and traces of fungicides were not visible, there could have been a systemic or residual effect on R. robini behaviour that is apparently unknown. In the field survey of populations of R. robini in corn plots, the number of mites collected was not affected by the Cry3Bb1 corn, the Gaucho seed treatment, or the insecticide applied to a subset of the farm plots. The combined results of these two experiments suggest there will be no negative impact of this new transgenic insecticidal corn on populations of the non-target mite, R. robini, commonly found in agricultural fields.

The wide-scale adoption of transgenic plants with below-ground parts expressing Bt toxins makes it imperative that both positive and negative effects on non-target organisms are investigated and compared with non-target effects of insecticidal treatments. We found no negative impact of either δ-endotoxin on R. robini in short- or longer-duration trials. We did not find a negative impact of Cry3Bb1 corn, or the alternative soil insecticide treatment (Force 3G) on mite population densities in the field. These results, combined with results from other studies, suggest that a shift from the use of soil insecticidal treatments to Bt toxin-expressing plant tissues for control of coleopteran pests of corn would have very little positive or negative effects on R. robini populations.
the results of this and other assessments of non-target impacts with studies on pest management efficacy and economic value will allow a thorough evaluation of this new technology.

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