Tritrophic Interactions Among *Bt* (Cry3Bb1) Corn, Aphid Prey, and the Predator *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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ABSTRACT The ability of the transgenic corn rootworm resistant corn (*Zea mays* L.) hybrid, MON 863, to affect the predator *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) through the consumption of corn-fed aphid (*Rhopalosiphum maidis*, Homoptera: Aphididae) prey was examined in the laboratory. Aphid weight was used as an index of prey quality. Larvae of *C. maculata* were reared to pupation on aphids that had consumed transgenic or nontransgenic (susceptible) corn plants. Larval duration, survivorship to pupation, postmortem adult dry weight (taken at 30 d after eclosion), adult mobility, and fecundity were compared for *C. maculata* between treatments. Fitness parameters of *C. maculata* were similar between transgenic and susceptible treatments, despite a 33% reduction in the weight of aphid prey reared on MON 863. Using immunostrip tests, Cry3Bb1 was detectable in the leaves of MON 863 but not in the susceptible plant, aphids, or *C. maculata* that were fed aphids. We conclude that transgenic corn that expresses Cry3Bb1 does not inflict acute or chronic degradations in fitness on individual *C. maculata* through aphid prey, but these results do not necessarily apply to other natural enemies, herbivores, or insect-resistant corn hybrids.

KEY WORDS biological control, predation, *Rhopalosiphum*, transgenic crop, trophic interactions

COMMERCIALLY AVAILABLE TRANSGENIC CORN hybrids express genes that encode the delta-endotoxins, or Cry toxins, from the entomopathogen, *Bacillus thuringiensis* (Berliner) (*Bt*). One commercial hybrid, MON 863, produces the toxin Cry3Bb1 and targets coleopteran pests, namely the corn rootworm species, *Diabrotica virgifera virgifera* LeConte and *D. barberi* Smith and Lawrence (EPA 2003). Toxins from *Bt* are relatively host specific within certain insect orders (e.g., Lepidoptera or Coleoptera), because susceptibility depends on an insect possessing specific gut physiological conditions and receptor sites (Knowles 1994, Garner et al. 1999). Nevertheless, insects outside of target groups have been reported to be susceptible to some Cry toxins of *Bt* (Hilbeck et al. 1998b), and the use of insect-resistant plants can have complex interactions with higher trophic levels within agroecosystems. It is important to the long-term sustainability of transgenic insecticidal crops that they are compatible with other management tactics, including biological control.

One ecological pathway through which transgenic insecticidal crops can interact with predators is through herbivorous prey. Prey-mediated effects on predator fitness by insect-resistant crops have been reported previously for transgenic crops in the laboratory (Hilbeck et al. 1998a, Birch et al. 1999, Ashouri et al. 2001, Bernal et al. 2002b, Burgess et al. 2002, Prütz and Dettner 2004). It is often unclear whether the observed deleterious effects of transgenic-insecticidal crops on secondary trophic levels result from actual toxicity of the insecticidal protein to the predator, from reductions in prey quality resulting from feeding on the transgenic plants, or a combination of both. In either case, the bottom-up cascade of deleterious effects of insecticidal proteins on nontarget herbivores and the predators that consume them can theoretically have important implications for the compatibility of genetically modified (GM) insect-resistant crops and biological control.

Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae) is a prevalent coccinellid predator in the corn-growing regions of the United States (Musser and Shelton 2003, Lundgren et al. 2004) and is an important natural enemy of several pests of corn, including the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae) (Wright and Laing 1980) and the eggs of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) (Phoofolo et al. 2001), and the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Cottrell and Yeargan 1998). In addition, *C. maculata* feeds on other aphid species, eggs of other pest species, and

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pollen. The primary focus of the effects of *Bt* corn on *C. maculata* has focused on pollen as a pathway for the toxin to reach the predator, and some hybrids of *Bt* corn pollen have been shown to have no toxicity to *C. maculata* (Pilcher et al. 1997, Duan et al. 2002, Lundgren and Wiedenmann 2002, Lundgren and Wiedenmann 2004). Prey-mediated effects of toxins expressed in insect-resistant corn have not been studied for this coccinellid species. Here, we tested the hypothesis that MON 863 corn has no deleterious effects on herbivorous aphids and the predator, *C. maculata*, that consumes them.

Materials and Methods

Corn and Insects. Seeds of corn that expresses the Cry3Bb1 protein (event MON 863; transgenic hybrid TXP 260B-F) and nontransgenic corn (hybrid EXP 260B; hereafter, susceptible) were provided by Monsanto Co. (St. Louis, MO) and were cultivated individually in 3.8-liter pots in separate greenhouse rooms, but under similar environmental conditions. Treatments were kept in separate rooms to avoid the movement of aphids between treatments. Each plant was fertilized weekly with 2.2 liters of aqueous Peters Professional General Purpose Fertilizer (20N, 10K, 20P; Scotts, Allentown, PA) at a concentration of 250 ppm.

Coleomegilla maculata adults were obtained from a laboratory colony that had been maintained at the Illinois Natural History Survey, Champaign, IL, continuously on artificial diet (based on diet 7 of Atallah and Newsom 1966) for 2 yr, with annual additions of field-collected specimens. Aphid colonies were reared on corn hybrids within the greenhouses; aphid individuals were not identified for feeding assays, although the aphids in these colonies were predominantly *Rhopalosiphum maidis* (Fitch) with a minority of *R. padi* L. The aphids were selected from the tassels and upper corn leaves, and because of the behavior of the two aphid species in our greenhouse, >90% of aphids were *R. maidis* (*R. padi* infested the lower one-third of the plant). Also, the aphids selected for experiments were primarily older nymphs and adults (apterous) of ≈1 mm and larger (dorsal view, anterior to posterior measurement). The biomass of living aphids was selected as an indicator of their relative quality as prey. Ten groups of 10 aphids were isolated from MON 863 and susceptible corn plants, and each group was weighed to the nearest 0.01 mg. The mean weights of the aphids fed the different corn were compared using analysis of variance (ANOVA) with JMP statistical software (JMP 3.2.6; SAS Institute, Cary, NC).

Serological Assays. Leaf tissue, prey, and predator larvae were subjected to qualitative serological tests to determine whether Cry3Bb1 could be detected up the trophic chain. Five samples (0.03 g each) of leaf tissue were removed from MON 863 and the susceptible hybrid. Five samples ($n = 40$ each) of randomly selected aphids were removed from the transgenic and susceptible hybrids and frozen (−10°C) until analyzed. To determine if predators contained Cry3Bb1 toxins after ingesting aphids, we provided third-instar *C. maculata* ($n = 3$) each with 40 aphids that had fed on either the transgenic or susceptible corn leaves for at least 24 h. After exposing the predators to prey for 24 h, the *C. maculata* were frozen (−10°C) until analyzed. After the leaves, prey, and predator larvae had been isolated, they were macerated in phosphate buffer; samples from the MON 863 treatments were suspended in 10 mM phosphate buffered saline with 0.05% Tween 20 (pH 7.4; product number P3563; Sigma). Solutions containing the sample tissues from MON 863 were subjected to immunostrip tests specific for Cry3Bb1 (provided by Monsanto Company).

Fitness Assays on *C. maculata*. Newly hatched first-instar *C. maculata* ($N = 82$) originating from multiple females (≈25) were systematically divided between two treatments so that offspring from each female were assigned randomly to treatments. Because of the time requirements involved in feeding aphids to the individual *C. maculata* larvae (especially later instars), five blocks were established over several weeks, between 5 and 13 larvae per treatment per block. Each larva was reared individually in a 30-ml plastic cup with a plastic lid, and a saturated cotton wick was provided. Experimental conditions were 14:10 (L:D) photoperiod, 27°C, and 20–35% RH. *Rhopalosiphum* were removed from infested MON 863 and susceptible corn and provided to the *C. maculata* larvae. Preliminary research revealed the approximate aphid-consumption rate by each *C. maculata* instar, and those data were used to assure that aphids were provided to the larvae in excess during the experiments. Fifteen, 20, 40, and 50 aphids were provided daily to first-, second-, third-, and fourth-instar *C. maculata*, respectively. Aphids that were not eaten were removed after 24 h, and fresh aphids were offered daily. The duration of each larval stadium and the entire larval stage, and the proportion of larvae surviving to pupation were recorded.

After pupae emerged, adult *C. maculata* were reared individually on artificial diet in 30-ml cups with plastic lids and water wicks. During the first 2 wk after the last individual emerged in each trial, beetles were subjected to mobility tests that consisted of measuring walking speeds and the durations until the beetles righted themselves when placed on their dorsum (flip-time) (Lundgren and Wiedenmann 2002). Each beetle was subjected to the walking-speed and flip-time tests three times sequentially without interruptions. After the mobility assays, the females were mated by exposing individual females to two randomly selected males from the *C. maculata* colony for 24 h. Approximately 10 d after the first mating, the females were remated under similar conditions. Females were isolated in their cups and were provided with a cardboard tube (2.5 cm long, 0.3 cm diameter) as an oviposition substrate. The mean number of fertilized eggs laid per female (measured as the number of eggs that hatched within 7 d) and the proportion of females that oviposited within 7 d and within 30 d after the first mating were recorded for each treatment. After the mobility

Table 1. Fitness parameters of *C. maculata* fed on aphids that were reared on MON 863 (transgenic) or susceptible (nontransgenic) corn

Fitness parameter	MON 863 (*n*)	Susceptible (*n*)
Larval duration (days)	9.68 ± 0.29 (43)	9.94 ± 0.13 (39)
Adult postmortem dry weights (mg)	4.50 ± 0.26 (43)	4.87 ± 0.32 (39)
Flip time (s)	15.10 ± 1.68 (28)	14.90 ± 1.66 (28)
Walking speed (cm/s)	4.71 ± 0.20 (28)	5.01 ± 0.27 (28)
7-d fecundity (eggs)	25.5 ± 8.97 (43)	18.35 ± 10.26 (39)
30-d fecundity (eggs)	128 ± 28.15 (43)	76.59 ± 29.56 (39)

Values are means ± SEM.

Flip times and walking speeds presented here are composites of the three observations for each individual; repeated-measures ANOVAs were used to make statistical inferences on the data. None of the differences between treatments were significant (Tukey-Kramer means comparisons; $\alpha = 0.05$).

and mating experiments, the postmortem dry weight was measured for each individual. Males and females were frozen within 7 d of the termination of the oviposition experiment and were dried at 60°C for 24 h.

Statistical Analyses. The duration of the larval stage, the numbers of eggs laid within 7 and 30 d of mating, and the postmortem dry weights were compared between the treatments with a standard least squares model that included block and treatment effects (JMP 3.2.6). Statistics did not reveal any differences in the fitness parameters in any of the blocks, and for analysis, data were pooled over blocks. Categorical data, the proportion of larvae that survived to adulthood, and the proportions of females that laid eggs within 7 and 30 d of mating were compared between treatments using a nominal logistic model (JMP 3.2.6). Finally, the flip times and walking speeds of *C. maculata* adults were compared between the treatments using repeated-measures ANOVAs (GLM Procedure; SAS Institute).

Results

We detected Cry3Bb1 in the leaf tissue of the MON 863 but not in the susceptible hybrid. Cry3Bb1 was not detected in any of the aphids reared on MON 863 plants or in coccinellids reared on MON863-fed aphids. Similarly, Cry3Bb1 was not detected in any aphids or coccinellids from the susceptible treatment.

Aphids weighed significantly less when fed MON 863 corn than when fed susceptible corn ($F_{1,18} = 38.43$, $P < 0.0001$). Mean ± SEM masses of the aphids were 0.22 ± 0.02 and 0.33 ± 0.01 mg in the MON 863 and susceptible treatments, respectively. The duration of *C. maculata* larval development and postmortem dry weights of adults were not significantly different between the susceptible and MON 863 treatments (duration: $F_{1,64} = 0.53$, $P = 0.47$; weight: $F_{1,66} = 0.63$, $P = 0.43$; Table 1). A similar proportion of *C. maculata* larvae survived to adulthood in these two treatments ($\chi^2_{1,80} = 0.24$, $P = 0.62$); 88 and 85% of larvae survived to adulthood in the susceptible and transgenic treatments, respectively. Also, flip times and walking speeds for *C. maculata* did not differ significantly among aphids fed MON 863 or susceptible corn tissue (flip time: $F_{1,54} = 0.01$, $P = 0.93$; walking speed: $F_{1,54} = 0.80$, $P = 0.37$; Table 1).

The percentages of females that laid eggs in 7 d were 18 and 44% in the susceptible and MON 863 treatments, respectively, and did not differ significantly between treatments ($\chi^2_{1,33} = 3.00$, $P = 0.08$). The percentages of females that laid eggs in 30 d were 35 and 67% in the susceptible and MON 863 treatments, respectively; however, the values from the treatments did not differ significantly ($\chi^2_{1,33} = 3.50$, $P = 0.06$). The numbers of eggs laid per female 7 and 30 d after mating also were not significantly different between the treatments (7 d: $F_{1,33} = 0.28$, $P = 0.60$; 30 d: $F_{1,33} = 1.59$, $P = 0.22$; Table 1). The 30-d fecundity for females that laid eggs was 217 ± 43 and 192 ± 27 eggs per female for the susceptible and MON 863 treatments, respectively.

Discussion

Our laboratory study did not reveal any deleterious effects on the fitness components examined of *C. maculata* who ingested aphid prey that had fed on transgenic corn expressing Cry3Bb1. The developmental rate, size, mobility, and fecundity of ladybird beetles were statistically similar among the transgenic and susceptible corn treatments. Those similarities were found despite reductions in the biomass of aphids that fed on the insect-resistant corn hybrids.

Our inability to detect the insecticidal toxins in the aphids fed MON863 corn is consistent with other published research. Cry toxins in *Bt* corn are not found at high levels in the phloem of lepidopteran-resistant corn hybrids, and consequently, phloem-feeding insects such as aphids do not generally contain appreciable levels of the toxin (Head et al. 2001, Raps et al. 2001, Dutton et al. 2002, Harwood et al. 2005). However, it should be noted that *Bt* toxins have been detected in the honeydew of aphids reared on transgenic rice (Bernal et al. 2002a), and transgenic corn may alter the long-term population dynamics of aphids under field conditions (Lumbierres et al. 2004). Although our immunostrips did not detect the Cry3Bb1 protein in aphids, and it has not been shown that feeding aphids encounter the toxin in this hybrid, a significant reduction in the biomass of aphids reared on MON 863 plants was detected. Even though research suggests that aphids will not encounter *Bt* insecticidal toxins from transgenic plants, an array of parasitoids and generalist predators in cornfields rely on aphids as prey, so the importance of these insects as prey/hosts necessitates that we include aphids in studies of tritrophic interactions of insect-resistant crops and natural enemies.

An important consideration in this tritrophic interaction is the cause of the reduction in size of aphids fed the transgenic plants. Essentially, the two corn hybrids that were compared are distinct genetic systems, regardless of how closely they are related to one another through parentage. Slight genetic differences result-

ing from the transformation process can have important phenotypic implications for the suitability of transgenic plants as food for aphids (Escher et al. 2000, Saxena and Stotzky 2001, Lumbierres et al. 2004). In this study, the inclusion of the *cry3Bb1* gene into MON 863 and the resulting expression of insecticidal protein is a major difference between the transgenic and susceptible lines, but identification of the actual factor or factors responsible for the reduction in aphid size requires further study. Simultaneously evaluating the suitability of multiple transgenic and susceptible corn lines on aphid growth and development would be a useful step in determining the level of variability inherent in corn hybrids.

The transgenic hybrid reduced aphid size, and further research should address the implications of reduced prey/host quality on the fitness of natural enemies within transgenic corn fields. In this study, we showed that reducing prey size by 33% was not sufficient to have measurable impacts on the fitness of the predator *C. maculata.* This may not be generally the case. In a comprehensive examination of tritrophic interactions among lepidopteran-resistant *Bt* corn, *Spodoptera littoralis* (Lepidoptera: Noctuidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae), a deleterious prey-mediated effect of transgenic corn on the immature survival of *C. carnea* was documented (Hilbeck et al. 1998a, 1999). Subsequent research determined that the reduction in lacewing survival could be explained by the reduced size (and likely reduced quality) of the corn-fed *S. littoralis* prey (Dutton et al. 2002, Romeis et al. 2004). However, diet-incorporated Cry proteins have direct deleterious effects on *C. carnea* survival (Hilbeck et al. 1998b), and so both prey quality and direct toxicity of Cry proteins may play a role in this system. Reductions in host quality resulting from feeding on transgenic plants also have been shown to affect parasitoid fitness. Aphids feeding on *Bt* potato were less suitable hosts for the parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae), which suffered higher mortality, lower adult weights, and lower fecundity when reared on them (Ashouri et al. 2001). Another parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae), had lower fitness when reared on *Chilo partellus* (Lepidoptera: Crambidae) hosts that had fed on *Bt* corn versus non-*Bt* corn (Prütz and Dettner 2004). Additional research is greatly needed to isolate the different ways that transgenic insecticidal crops can influence insectivorous arthropods and biological control tritrophically.

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References Cited

Ashouri, A., D. Michaud, and C. Cloutier. 2001. Recombinant and classically selected factors of potato plant resistance to the Colorado potato beetle, *Leptinotarsa decemlineata,* variously affect the potato aphid parasitoid *Aphidius nigripes.* BioControl. 46: 401–418.

Atallah, Y. H., and L. D. Newsom. 1966. Ecological and nutritional studies on *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). I. The development of an artificial diet and a laboratory rearing technique. J. Econ. Entomol. 59: 1173–1178.

Bernal, C. C., R. M. Aguda, and M. B. Cohen. 2002a. Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis.* Entomol. Exp. Applic. 102: 21–28.

Bernal, J. S., J. G. Griset, and P. O. Gillogy. 2002b. Impacts of developing on Bt maize-intoxicated hosts on fitness parameters of a stem borer parasitoid. J. Entomol. Sci. 37: 27–40.

Birch, A.N.E., I. E. Georhegan, M.E.N. Majerus, J. W. McNicol, C. A. Hackett, A.M.R. Gatehouse, and J. A. Gatehouse. 1999. Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. Molec. Breeding. 5: 75–83.

Burgess, E.P.J., G. L. Lövei, L. A. Malone, I. W. Nielson, H. S. Gatehouse, and J. T. Christeller. 2002. Prey-mediated effects of the protease inhibitor aprotinin on the predatory carabid beetle *Nebria brevicollis.* J. Insect Physiol. 48: 1093–1101.

Cottrell, T. E., and K. V. Yeargan. 1998. Effect of pollen on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. Environ. Entomol. 27: 1402–1410.

Duan, J. J., G. Head, M. McKee, T. Nickson, J. W. Martin, and F. S. Sayegh. 2002. Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a nontarget beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae). Entomol. Exp. Applic. 104: 271–280.

Dutton, A., H. Klein, J. Romeij, and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea.* Ecol. Entomol. 27: 441–447.

Environmental Protection Agency [EPA]. 2003. *Bacillus thuringiensis* Cry3Bb1 protein and the genetic material necessary for its production (vector ZMIRL13L) in event MON 863 corn fact sheet. EPA, Washington, DC.

Escher, N., B. Käch, and W. Nentwig. 2000. Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porciello scaber* (Crustacea: Isopoda). Basic Appl. Ecol. 1: 161–169.

Garner, K. J., S. Hiremath, K. Lehtoma, and A. P. Valaitis. 1999. Cloning and complete sequence characterization of two gypsy moth aminopeptidase-N cDNAs, including the receptor for *Bacillus thuringiensis* Cry1Ac toxin. Insect Biochem. Molec. Biol. 29: 527–535.

Harwood, J. D., W. G. Wallin, and J. J. Obrycki. 2005. Bt-toxin flows through the arthropod food chain. Molec. Ecol. 14: 2815–2823.

Head, G., C. R. Brown, M. E. Groth, and J. J. Duan. 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Applic. 99: 37–45.

Hilbeck, A., M. Baumgartner, P. M. Fried, and F. Bigler. 1998a. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27: 480–487.

Hilbeck, A., W. J. Moar, M. Pusztai Carey, A. Filippini, and F. Bigler. 1998b. Toxicity of the *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae) using diet-incorporated bioassays. Environ. Entomol. 27: 1255–1263.

Hilbeck, A., W. J. Moar, M. Pusztai Carey, A. Filippini, and F. Bigler. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea.* Entomol. Exp. Applic. 91: 305–316.

Knowles, B. H. 1994. Mechanisms of action of *Bacillus thuringiensis* insecticidal δ-endotoxins. Adv. Insect Physiol. 24: 275–308.

Lumbierres, B., R. Albajes, and X. Pons. 2004. Transgenic Bt maize and *Rhopalosiphum padi* (Hom., Aphididae) performance. Ecol. Entomol. 29: 309–317.

Lundgren, J. G., and R. N. Wiedenmann. 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn does not affect the fitness of the non-target species, Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae). Environ. Entomol. 31: 1213–1218.

Lundgren, J. G., A. A. Razzak, and R. N. Wiedenmann. 2004. Population responses and feeding behaviors of the predators *Coleomegilla maculata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Environ. Entomol. 33: 958–963.

Lundgren, J. G., and R. N. Wiedenmann. 2004. Nutritional suitability of field corn pollen as food for the predator, *Coleomegilla maculata* (Coleoptera: Coccinellidae). J. Insect Physiol. 50: 567–575.

Musser, F. R., and A. M. Shelton. 2003. Factors altering the temporal and within-plant distribution of coccinellids in corn and their impact on potential intra-guild predation. Environ. Entomol. 32: 575–583.

Phoofolo, M. W., J. J. Obrycki, and L. C. Lewis. 2001. Quantitative assessment of biotic mortality factors of the European corn borer (Lepidoptera: Crambidae) in field corn. J. Econ. Entomol. 94: 617–622.

Pilcher, C. D., J. J. Obrycki, M. E. Rice, and L. C. Lewis. 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26: 446–454.

Prütz, G., and K. Dettner. 2004. Effect of Bt corn leaf suspension on food consumption by *Chilo partellus* and life history parameters of its parasitoid *Cotesia flavipes* under laboratory conditions. Entomol. Exp. Applic. 111: 179–187.

Raps, A., J. Kehr, P. Gugerli, W. J. Moar, F. Bigler, and A. Hilbeck. 2001. Detection of Cry1Ab in phloem sap of *Bacillus thuringiensis* corn and in the selected herbivores *Rhopalosiphum padi* (Homoptera: Aphididae) and *Spodoptera littoralis* (Lepidoptera: Nocutidae). Molec. Ecol. 10: 525–533.

Romeis, J., A. Dutton, and F. Bigler. 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). J. Insect Physiol. 50: 175–183.

Saxena, D., and G. Stotzky. 2001. *Bt* corn has a higher lignin content than non-*Bt* corn. Am. J. Botany. 88: 1704–1706.

Wright, E. J., and J. E. Laing. 1980. Numerical response of coccinellids to aphids in corn in southern Ontario. Can. Entomol. 112: 977–988.

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