Effects of Transgenic *Bacillus thuringiensis* Corn and Permethrin on Nontarget Arthropods[1]

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ABSTRACT Planting of insect-resistant transgenic corn has led to concerns about potential effects on nontarget arthropods. Various sampling methods (visual counts, yellow sticky cards, pitfall traps) were used at 7-to 10-day intervals to test for possible changes in abundance for common nontarget arthropods caused by transgenic or insecticide-based pest management. Large-scale field studies arranged in randomized complete blocks were conducted in 2001 and 2002 in central Iowa. Treatments included Bt corn (Pioneer 34M95, event MON810), a nonBt isoline (Pioneer 34M94), and the nonBt isoline treated with permethrin (Pounce 1.5G®). There was high variation in population estimates for the majority of the arthropods collected. Consequently, only data from taxa with coefficients of variation ≤75 using a particular sampling method were used in statistical analysis. Insecticide applications negatively impacted populations of arachnids (Araneae and Opiliones), Cantharidae, Elateridae and *Macrocentrus cingulum* (Hymenoptera: Braconidae). In the transgenic treatment, fewer *M. cingulum* and Nitidulidae were present, presumably because of a lack of *Ostrinia nubilalis* (Lepidoptera: Crambidae), which serve as hosts for *M. cingulum* and provide habitat for Nitidulidae, which are known to frequent *O. nubilalis* tunnels. However, application of a conventional insecticide for *O. nubilalis* control had a broader impact on populations of various nontarget arthropods. These results are consistent with other studies that suggest nontarget impacts resulting from insecticides may be greater than those caused by Bt corn.

KEY WORDS nontargets, coefficient of variation, arthropods, Bt corn

The European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) can cause severe economic injury to corn and has been historically controlled by chemicals, host-plant resistance, and biological control (Bergman et al. 1985). In recent years, *O. nubilalis* control has been achieved by planting genetically modified insecticidal corn. Several varieties of corn have been modified by insertion of specific genes to continuously express the crystalline (Cry) proteins of *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) (Bt). These proteins dis-

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rupt the digestive systems of some insects, leading to gut paralysis and death (Tanada & Kaya 1993). Strains of Bt-producing different Cry proteins have selective toxicity because of the conditions necessary to solubilize, activate, and bind the proteins. The earliest varieties of Bt corn were modified by the insertion of lepidopteran-specific genes for *O. nubilalis* control. Because Bt must be ingested to have toxic effects, lepidopteran-specific Bt corn should kill only *O. nubilalis* or other susceptible Lepidoptera feeding on corn, leaving the remainder of the arthropod community unharmed.

However, because Bt corn has been genetically modified and has insecticidal properties, there are still questions concerning its safety regarding nontarget organisms. The potential for negative effects on nontarget arthropods has been an area of active research after a report that monarch larvae *Danaus plexippus* (L.) (Lepidoptera: Danaidae) experienced toxicity as a result of Bt pollen (Losey et al. 1999). These findings led to additional research into nontarget effects of Bt corn, including community-level responses (Dively 2005, Prasifka et al. 2005), effects of Bt pollen consumption (Hellmich et al. 2001, Dively et al. 2004), Bt plant material in the soil (Al-Deeb et al. 2003), and effects of Bt on predators and parasitoids (Dutton et al. 2003, Bhatti et al. 2005). Because of the complexity of these relationships and the possibility of additional unknown interactions, in-field studies are needed to assess what risks Bt corn may pose to nontarget arthropods.

Determining the effects of Bt crops on nontarget arthropods is complicated because clear protocols for selecting an experimental design, which taxa to sample, sampling methods, and analysis of results have not been established. The experimental design used must address plot size, replication, nontarget groups to be sampled, and a method for estimating nontarget group population size. Plot size and replication are important because inadequately sized plots can make it difficult to find statistically significant differences (Prasifka et al. 2005) and insufficient replication leads to decreased statistical power (Lopez et al. 2005). The choice of taxa can be narrow (e.g., sampling predators and parasitoids of the target pest [Pilcher et al. 2005], or all members of a group such as Collembola [Bitzer et al. 2005]), or broad (census-type sampling, i.e., sampling all arthropods present in the field [Dively 2005]). Methods for estimating population size should be suited to the arthropods being sampled; although both ground and ladybird beetles are coleopterans, general differences in locomotion (crawling versus flying) and microhabitat (ground versus foliage) suggest different sampling methods are appropriate for each type of beetle.

The objective of this study was to determine whether nontarget arthropods in Iowa corn fields were impacted by Bt corn and conventional insecticide applications using a standard randomized complete block experimental design and various sampling methods. The arthropods recorded were common in corn and could be identified with taxonomic certainty without requiring external professional expertise. In an attempt to include many potential avenues for nontarget effects, arthropods that occupied various ecological roles (predator, parasitoid, herbivore, and fungivore) were included. Rather than using a broad (community-wide) analysis or looking for effects on a narrow group of arthropods, we sampled many of the nontarget taxa using three common methods of population estimation, but restricted our data analysis to those groups that met a minimum sampling standard based on abundance and variability.

Materials and Methods

Field plots. Plots at the Sorenson farm, 13 km west of Ames, Iowa, were located within a single 198 × 190-m field that had been under corn-soybean rotation for 6 years. A randomized complete block experimental design with three replications was used. Replicates (61 × 198 m) were separated by 3-m alleyways, and plots were surrounded by five border rows (4 m) of nonBt isoline corn. Each replicate was divided into three treatments that were 80 rows (61 m) wide. Treatments included Bt corn (Pioneer 34M95, event MON810), an untreated nonBt isoline (Pioneer 34M94), and the nonBt isoline treated with permethrin (Pounce 1.5G®, permethrin, FMC Agricultural Products, Philadelphia, Pennsylvania), which is approved for use against first- and second-generation *O. nubilalis*. Corn was planted 16 May 2001 and 9 May 2002. Insecticide applications were made to coincide with the flight of *O. nubilalis* adults on 2 August (2001), 27 June, and 21 August (2002) at the rate of 11,340 g/ha. The experiment was designed to receive an insecticide application to coincide with both generations in 2001 and 2002, but weather conditions did not allow for first-generation applications in 2001. In 2002, treatments were planted in the same locations within the field as in 2001. Within each plot (80 rows), there were six rows designated for trapping, each containing five trapping stations (pitfall and yellow sticky cards) 4.5 m apart for a total of 10 traps per treatment and 30 per replicate. During each sampling period, two rows (11.4 m apart) of every treatment were sampled. Traps were operated for 7–10 days at 2-week intervals from June through October (2001) or September (2002).

Pitfall traps. Pitfall trapping was conducted as described by Lopez et al. (2005). After collection, pitfall traps were kept at 4°C until processed. To remove soil and antifreeze, the trap contents were first sifted through a 2.36-mm mesh screen. Large (more than 3 mm in length) arthropods were removed, rinsed with tap water, and placed in 70% ethanol. The remaining contents were then rinsed with tap water over a 45-μm mesh screen to remove fine soil particles, and the arthropods, along with the larger soil particles, were placed in a flat-topped modified separatory funnel filled with a saturated (45%) sugar solution. Arthropods floated in the sugar solution, whereas soil settled out and was removed. The contents of the separation funnel were poured through a 45-μm screen, rinsed with 70% ethanol, and placed into 70% ethanol along with the large arthropods removed previously. Processed samples were held at 4°C until arthropods could be identified.

Each sample was poured into a shallow 9-cm Petri plate. Relatively large arthropods were removed and identified and the remaining alcohol (containing smaller arthropods) was poured into a Büchner funnel lined with filter paper and vacuum-filtered. The filter paper was examined under a dissecting microscope and arthropods identified. Total numbers of the arthropods that were both small and numerous were estimated by counting the number that fell more than halfway into a marked area (10% of the total) of the filter paper and extrapolating. Numbers of aphids and collembola were commonly estimated in this way, and occasionally Staphylinidae and arachnids when they were numerous.

Sticky traps. Yellow 7.6 × 12.7 cm sticky traps (Olson Products, Medina, Ohio) with one sticky side exposed were placed within corn rows attached to 1.2-m ×1.0-cm unpainted bamboo stakes. They were attached to the stake with clothes pins with cards perpendicular to the ground and at right angles to the corn row. Sticky traps were placed at canopy height until the corn reached 1 m, after which all sticky traps were placed at the top of each bamboo stake. Sticky traps collected from the field were placed into individual 15- × 20-cm clear plastic bags with the sticky side of the card adhered smoothly to one side of the bag. The bags were held at 4°C until the cards were examined (through the plastic bags) and the arthropods identified.

Visual plant examinations. Ten plants within each treatment were selected at random and visually inspected for arthropods. Plants were destructively sampled to account for arthropods in the leaf collars and ear tips. Starting at the base of the plant, the stalk, top and bottom of the leaves, leaf collars, ear tips, silks, and tassels were examined, and the arthropods recorded. At the beginning of the season, each plant was examined by a single person, but as the corn grew taller than 1 m, plants were sampled by a pair of individuals to reduce the number of arthropods missed as they flew away. Sampling occurred every 7–10 days from mid-June to mid-October 2001 and early June to late September 2002.

Data analysis. Individual taxa with coefficients of variation (CV = 100 × standard deviation/mean) of 75 or less in the untreated control plots, for a given sample method (pitfall trap, sticky card, visual inspection) on the sample date immediately after insecticide application in 2001 or 2002 (either sample date) were selected for analysis. The CV is a scale-independent measure of variability among samples (Snedecor & Cochran 1989), allowing it to be used as a measure of variability for arthropods, which differ greatly in mean abundance. Coefficient of variation has been successfully used to evaluate the suitability of test systems for evaluating nontarget effects on plants (Reuter et al. 2002). Duan et al. (2006) noted that nontarget arthropods sampled with a CV < 100 tended to have greater statistical power. Insecticide treatment was included as a positive control for comparative risk assessment (Romeis et al. 2006). Therefore, we focused analysis on data collected immediately after insecticide applications. These dates were believed most likely to show impacts on nontarget arthropod populations. Taxa with a CV > 75 on the sample date immediately after insecticide applications were not analyzed because the likelihood of appropriately detecting treatment effects was considered to be similar to the chance of committing an error (particularly Type I errors; Snedecor & Cochran 1989). Although the use of a repeated measures analysis was considered, it was not used because these analyses would include data before and between insecticide applications, during which time arthropod populations could recover. Data were analyzed with analysis of variance using the General Linear Models Procedure and Tukey's multiple range test was used to separate means (SAS Institute 1999). Data were normalized by a $\log_{10}(x + 1)$ transformation where x is the mean number of individuals per plot for a taxon (using a given sampling method). Because of the relatively greater consequence of failing to detect changes in nontarget abundance, the significance level used for all statistical analysis was $P \leq 0.10$. A companion paper focuses on the impacts of Bt corn and insecticide treatment on the populations of individual ground beetle species (Lopez et al. 2005).

Results

Data on several taxa were collected from more than one sampling-method [e.g., Arachnida (Araneae and Opiliones), corn rootworm adults (*Diabrotica* spp.) (Coleoptera: Chrysomelidae), and leafhoppers (Cicadellidae)]. However, despite the large number of taxon × sampling method combinations used, the number of taxa with a CV ≤ 75 or less on a sample date immediately after insecticide application was modest. A complete list of the taxa sampled using each method, including the taxon × method combinations with a CV ≤ 75 is presented in Table 1.

Bt effects. There were few significant differences in nontarget arthropod populations resulting from Bt corn. In 2001, there were significantly fewer *Macrocentrus cingulum* Reinhard (Hymenoptera: Braconidae) captured on yellow sticky cards in the Bt corn plots than in the isoline control plots ($F = 18.93$; df = 2, 6; $P = 0.003$; Table 2). There were clear reductions in the mean numbers of *M. cingulum* captured on sticky cards in the Bt corn plots in 2001 and 2002 compared with the isoline (Fig. 1). However, in 2002 the CV for *M. cingulum* captures was too high to warrant analysis. Nitidulidae was the only taxon significantly impacted in the Bt corn plots in 2002 (Fig. 2). There were significantly fewer Nitidulidae in pitfall traps in the Bt corn than in the isoline control plots after the second insecticide application in 2002 ($F = 6.08$; df = 2, 6; $P = 0.04$; Table 2).

Insecticide effects. In 2001, visual inspections indicated that insecticide-treated plots contained fewer Arachnida ($F = 16.80$; df = 2, 6; $P = 0.004$; Table 2) but significantly more Aphidae ($F = 4.25$; df = 2, 6; $P = 0.07$; Table 2) compared with isoline control plots. Fewer *M. cingulum* were captured on yellow sticky cards ($F = 18.93$; df = 2, 6; $P = 0.003$; Table 2) and fewer Cantharidae larvae were found in pitfall traps ($F = 3.36$; df = 2, 6; $P = 0.10$; Table 2) in the insecticide treated plots compared with the isoline control plots.

After the first insecticide application in 2002, fewer Arachnida ($F = 4.95$; df = 2, 6; $P = 0.05$; Table 2) and Elateridae adults ($F = 11.90$; df = 2, 6; $P = 0.008$; Table 2) were collected from pitfall traps in insecticide-treated plots than the isoline control plots. Also, more adult Staphylinidae were found on yellow sticky cards in the insecticide-treated plots than in the untreated control ($F = 4.25$; df = 2, 6; $P = 0.07$; Table 2). After the second insecticide application in 2002, fewer Arachnida were found in visual inspections ($F = 3.75$; df = 2, 6; $P = 0.09$), and captured on yellow sticky cards ($F = 4.79$; df = 2, 6; $P = 0.06$; Table 2) in insecticide-treated plots than the control plots on the second sample date.

Discussion

Overall, there were few significant differences detected as the result of Bt corn or insecticide treatment. In 2001, there were two taxa from the visual plant inspections (Aphidae, Arachnida), one from yellow sticky card captures (*M. cingulum*), and one from pitfall traps (Cantharidae) that were significantly affected by Bt or insecticide treatment. In 2002, there was one taxon with a significant difference in the number of individuals observed from visual plant inspections (Arachnida), two from yellow sticky card captures (Arachnida, Staphylinidae) and three from pitfall traps (Arachnida, Elateridae, Nitidulidae). The two taxa that were significantly impacted as the result of Bt corn in this study are both known to be closely associated with *O. nubilalis*, and the observed decrease in their populations was likely caused by the absence of *O. nubilalis* in the Bt plots. These results suggest drastically decreasing the target pest population (as is the case in Bt-corn) can effect multitrophic level interactions in corn that occur with normal pest populations.

Table 1. List of nontarget arthropods sampled from near Ames, Iowa and analyzed in 2001–2002.

Nontarget group	Life stages	Pitfall	Sticky trap	Visual count
Aleyrodidae	Adults		A[a]	A
Alticinae	Adults			A
Aphidae	Nymphs, adults	B	B	B[b]
Arachnida (Araneae and Opiliones)	Nymphs, adults	B	B	B
Cantharidae	Adults	B	A	A
Cantharidae	Larvae	A		
Carabidae	Larvae	B		
Chilopoda	Larvae, adults	B		
Chrysopidae	Eggs			B
Chrysopidae	Larvae	B		A
Chrysopidae	Pupae			A
Chrysopidae	Adults		B	A
Cicadellidae	Nymphs, adults		B	B
Cicindellidae	Adults	A		
Coccinella septempunctata L.	Adults		A	A
Coccinellidae[c]	Eggs			A
Coccinellidae[c]	Larvae			A
Coccinellidae[c]	Pupae			B
Coleomegilla maculata DeGeer	Adults		A	A
Collembola	Nymphs, adults	B		
Cycloneda munda (Say)	Adults		B	A
Diabrotica spp.	Adults		A	B
Elateridae	Adults	B	A	A
Formicidae	Adults	A		A
Gryllidae	Nymphs, adults	B		
Harmonia axyridis (Pallas)	Adults		A	A
Hemerobiidae	Adults		A	A
Hippodamia convergens Guerin-Meneville	Adults		A	A
Hippodamia tredecimpunctata (L.)	Adults		A	A
Lampyridae	Adults	A	B	
Macrocentrus cingulum Reinhard	Adults		B	
Nitidulidae	Adults	B		B
Orius insidiosus (Say)	Adults		B	B
Pentatomidae	Eggs			A
Pentatomidae	Nymphs, adults	A	A	A
Scarabeidae	Adults	A		
Staphylinidae	Adults	B	B	A

[a]A indicates the nontarget group was sampled using the method indicated by column heading.
[b]B indicates the taxon was sampled using the method indicated by column heading and had a CV ≤ 75 on at least one selected sample date. These data were subsequently analyzed with analysis of variance, and those with a statistical difference between treatments on any sample date are presented (Table 2).
[c]All species combined for this life stage.

Table 2. Mean abundance (± SD) of taxa for which Bt or permethrin treatments differed from the untreated isoline, collected near Ames, Iowa, 2001–2002.

Sampling method	Taxon	Treatment	2001	2002	
			August 2[a]	June 27	August 21
Visual counts	Aphidae	Transgenic	0.46 (0.47)b	NA[b]	0.93 (0.90)a
		Insecticide	1.80 (1.28)a		1.50 (1.40)a
		Isoline	0.30 (0.10)b		0.83 (0.40)a
	Arachnida	Transgenic	0.70 (0.20)a	0.23 (0.32)a	0.70 (0.17)ab
		Insecticide	0.10 (0.10)b	0.10 (0.10)a	0.63 (0.15)b
		Isoline	0.63 (0.15)a	0.17 (0.06)a	0.93 (0.06)a
Sticky traps	Arachnida	Transgenic	NA	NA	1.05 (0.47)ab
		Insecticide			0.63 (0.15)b
		Isoline			1.37 (0.06)a
	Macrocentrus cingulum	Transgenic	0.49 (0.16)b	NA	NA
		Insecticide	0.90 (0.20)b		
		Isoline	1.96 (0.54)a		
	Staphylinidae	Transgenic	NA	0.11 (0.11)b	0.21 (0.18)a
		Insecticide		0.34 (0.13)a	0.20 (0.10)a
		Isoline		0.17 (0.05)b	0.36 (0.06)a
Pitfall traps	Arachnida	Transgenic	16.53 (9.01)a	10.42 (4.17)a	35.00 (15.00)a
		Insecticide	8.00 (4.95)a	4.24 (2.18)b	18.83 (5.69)a
		Isoline	16.37 (8.12)a	14.78 (8.61)a	35.87 (16.49)a
	Cantharidae	Transgenic	1.43 (1.07)a	NA	NA
		Insecticide	0.23 (0.06)b		
		Isoline	3.27 (2.60)a		
	Elateridae	Transgenic	NA	2.57 (0.61)a	NA
		Insecticide		0.29 (0.51)b	
		Isoline		4.52 (2.94)a	
	Nitidulidae	Transgenic	1.50 (1.13)a	3.13 (2.50)a	0.47 (0.06)b
		Insecticide	3.10 (1.75)a	2.02 (0.85)a	1.40 (0.75)ab
		Isoline	1.90 (0.56)a	5.06 (2.81)a	1.80 (0.69)a

[a]Means ($n = 3$) from the same taxa and year followed by the same letter are not significantly different ($P < 0.10$).
[b]The coefficient of variation was too large to warrant analysis.

Macrocentrus cingulum is a specialist larval parasitoid of *O. nubilalis* imported from Europe and the Orient (Baker et al. 1949) that is well synchronized with *O. nubilalis* larval presence in the field (Bruck & Lewis 1998) and remains the most common larval parasitoid in Iowa (Lewis 1982, Bruck & Lewis 1998, Bruck & Lewis 1999). Bt corn provides 85–100% control of *O. nubilalis*, depending on the genetic event (Ostlie et al. 1997). A presumed lack of *O. nubilalis* larvae in the Bt corn plots is likely the cause for the reduction in *M. cingulum* captures. Because *M. cingulum* actively searches for and finds hosts based on olfactory cues (Ding et al. 1989), with no *O. nubilalis* larvae present, *M. cingulum* would not be likely to remain in fields of Bt corn. Although we did not directly sample *O. nubilalis* in the experimental field, we know from capture data from two light traps in central Iowa that in 2001 and 2002, *O. nubilalis* populations were below

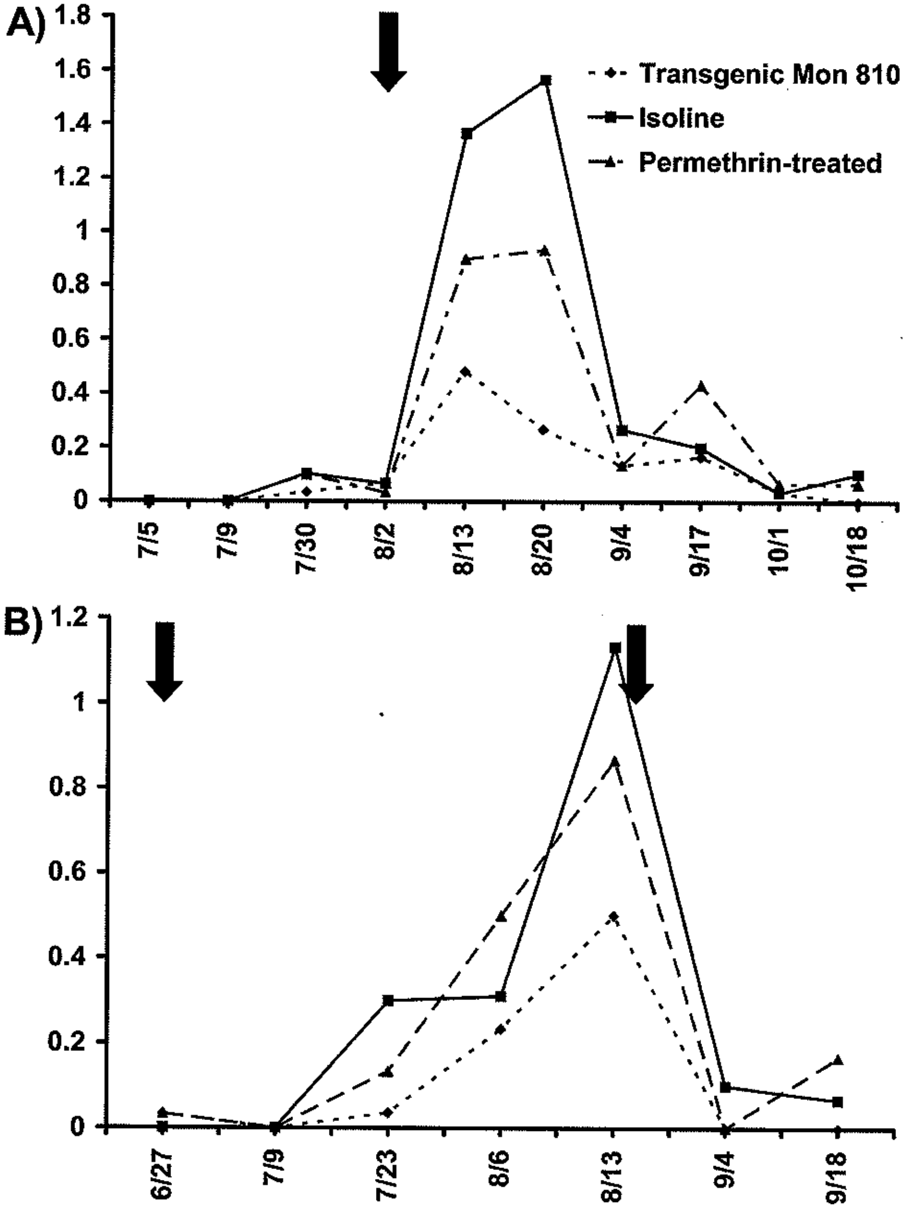
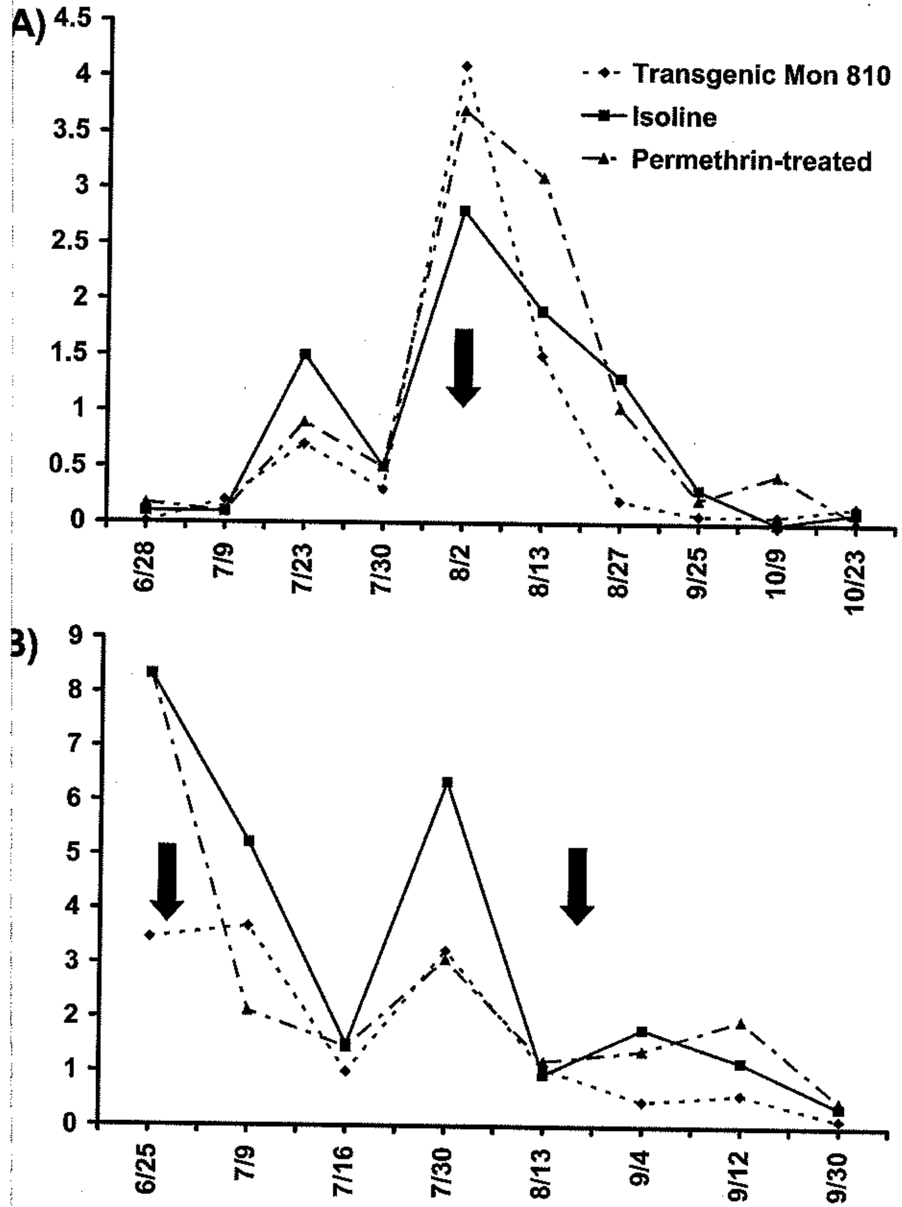


Fig. 1. Mean number of *Macrocentrus cingulum* (Hymenoptera: Braconidae) captured on sticky cards in 2001 (A) and 2002 (B). Timing of insecticide applications noted by downward arrow. Traps were placed into the field 24 h after insecticide application, and removed from the field 7–10 days later.



ig. 2. Mean number of Nitidulidae captured in pitfall traps in 2001 (A) and 2002 (B). Timing of insecticide applications noted by downward arrow. Traps were placed into the field 24 h after insecticide application, and removed from the field 7–10 days later.

average when compared with the previous ten years (Hellmich 2006), so any minor effect detected in these years would only be more pronounced in years of greater *O. nubilalis* populations. Pilcher et al. (2005) also found a 29–60% reduction in *M. cingulum* numbers in Bt corn compared with nonBt corn. *Macrocentrus cingulum* is preferentially recruited to and increases over time in nonBt corn plots (Pilcher et al. 2005). We also observed an increase in *M. cingulum* numbers over time in the isoline control plots in 2001 and 2002 (Fig. 1).

Several species of Nitidulidae have long been associated with corn (Vinal & Caffery 1919). Many Nitidulidae are fungivores and have frequently been found in tunnels in corn made by *O. nubilalis* larvae (McCoy & Brindley 1961). The beetles likely feed on the fungi growing on the plant exudates in these tunnels. Nitidulidae adults are also attracted to corn ears that have been damaged by other insects (Dowd 2000). Although we did not quantify the *O. nubilalis* infestation in these plots, a lack of *O. nubilalis* tunneling in the Bt corn would make those plots less attractive to Nitidulidae. Our data, along with reports from Daly & Buntin (2005), support the hypothesis that Bt corn is less attractive to Nitidulidae. The reduction in attractiveness of Bt corn to Nitidulidae may also be attributable to the reduction of exposed kernels (Dowd 2000).

Many studies have shown that crop treatment with a synthetic insecticide against a target pest population has broad toxicity against many groups of nontarget arthropods (Cherry & Pless 1971, Croft 1994, Boyd & Boethel 1998, Amalin et al. 2001, Duan et al. 2004) as well as resulting in pest resurgence (Pedigo 2002). *Ostrinia nubilalis* is a chronic bivoltine pest of corn in central Iowa (Mason et al. 1996), and several different insecticides are labeled for *O. nubilalis* control (Mason et al. 1996, Catangui & Berg 2002), including Pounce 1.5G. The use of insecticides may cause greater harm to nontarget organisms than Bt corn, which is why we believe that the safety of Bt corn to nontarget arthropods must be made in comparison with that of insecticide application. Bhatti et al. (2005) found that foliar permethrin application significantly reduced arachnid populations compared with isoline control plots. Dively (2005) also found that foliar insecticide application had a more consistent and sustained impact on nontarget arthropod populations than Bt corn.

In this study, we attempted to sample and test a wide variety of taxa for nontarget effects using a standard experimental design. An insecticide treatment was included to provide a positive control to verify that differences between treatments were detectable; however, only a few significant effects of insecticide treatment were detected. Although the sample dates in these studies were focused on those following insecticide applications, it may be that enough time elapsed for most arthropods to repopulate insecticide treated plots from surrounding untreated control and Bt plots. The daily dispersal rate of some of the most commonly encountered nontarget arthropods in our study is 25–32 m/day (Prasifka et al. 1999). In addition, because pitfall and sticky traps were active in the field for 7–10 days at a time, immediate effects of the insecticide applications may have been diluted between collections. This may have been a limitation in the insecticide treated plots, but because event MON810 continuously expresses the Cry1Ab protein, we believe that the low number of differences observed between Bt corn and the isoline control plots is realistic.

Our observations of few detrimental impacts to nontarget arthropods caused by Bt corn are similar to those seen by others (Daly & Buntin 2005, Dively 2005, Lopez et al. 2005, Romeis et al. 2006). Al Deeb et al. (2001) reported no significant effects of Bt corn on *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). In addition, Al-Deeb et al. (2003) reported no deleterious effects of Bt corn on beneficial and other nontarget arthropods from Bt corn expressing Coleopteran-specific (Cry3Bb1) protein. Orr & Landis (1997) also saw no significant differences in the numbers of three predators and an egg parasite of *O. nubilalis* in Bt corn. Ingesting prey fed Coleopteran-specific Bt proteins did not adversely affect *C. maculata* (Riddick & Barbosa 1998). Al-Deeb & Wilde (2003) also found no significant differences in *C. maculata* populations between Bt corn plots and plots of its nonBt isoline. Dively (2005) found the community disturbances caused by Bt corn were significantly less profound than those caused by insecticide application. In laboratory tests, the fitness of *C. maculata* feeding on transgenic corn pollen (Cry3Bb) was not significantly different than of those fed nontransformed pollen (Lundgren & Wiedenmann 2002). Losey et al. (1999) did find reduced *D. plexippus* leaf consumption, larval survival, and larval weight when fed milkweed leaves dusted with Bt pollen. However, the pollen dosage used in this study was not controlled and may not have accurately mimicked the pollen distribution rates in the field (Hellmich et al. 2001). For some nontarget arthropods (particularly generalist predators), corn planting date and subsequent crop phenology can have a stronger influence on their population size than Bt-corn (Pilcher et al. 2005). Shelton et al. (2002) proposed that regardless of the technique used to reduce a pest population (Bt plants, insecticides, biological control, etc.) there will be an impact on the overall biological community.

In summary, only two of the taxa sampled experienced a significant decrease in Bt corn. It is likely that a lack of *O. nubilalis* in the Bt plots directly and indirectly impacted *M. cingulum* and Nitidulidae via host elimination and removal of preferred habitat, respectively. In contrast, five taxa experienced significant declines in the insecticide treated plots. These results are consistent with several other studies that suggest nontarget impacts because insecticides may be greater than those caused by Bt corn.

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