EFFECTS OF BT TRANSGENIC AND CONVENTIONAL INSECTICIDE CONTROL ON THE NON-TARGET NATURAL ENEMY COMMUNITY IN SWEET CORN

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INTRODUCTION

Sweet corn containing a gene from the bacterium *Bacillus thuringiensis* (referenced as *B.t.* or Bt for the organism, but as BT for its extracted genes) was introduced commercially in 1996, as Attribute hybrids by Syngenta Seeds. The pesticide-incorporated plants contain Cry1Ab and PAT marker transgenes inserted by traditional breeding using event BT11 in transgenic field corn. Control efficacy is remarkably high, preventing virtually 100% of the damage caused by European corn borer (*Ostrinia nubilalis* Hübner) and providing more than 95% control of the corn earworm (*Helicoverpa zea* Boddie) and fall armyworm (*Spodoptera frugiperda* Smith). Use of Bt sweet corn hybrids can reduce the number of insecticide applications by 75 to 100%.

Like any insect control technology, Bt transgenic control may present a risk to the natural enemy community. Although tiered laboratory tests indicate no acute adverse effects on a suite of individual non-target organisms (Sims 1995, 1997), few studies have been conducted at the community level to assess tritrophic effects. There are many pathways by which transgenic sweet corn can interact both directly and indirectly with target and non-target organisms at different trophic levels within the crop system, as well as in habitats outside the crop in the surrounding landscape (Schuler *et al.* 1999ab). A reduction in host or prey populations, indirect contact with Cry protein by feeding on intoxicated organisms, feeding directly on plant parts (e.g., pollen), or changes in plant chemical cues could all have adverse effects on natural enemies. Conversely, reductions in insecticide use resulting from planting Bt sweet corn should be beneficial to natural enemies (Betz *et al.* 2000). Predators and parasitoids allowed to persist in Bt fields may aid in controlling secondary non-target pests. Thus, an assessment of the ecological risks of Bt sweet corn on non-target organisms should include a comparison with the risks of conventional control methods. Furthermore, risks should be assessed in a field experiment large enough to represent real world conditions and involve a broad taxonomic range of organisms at the community level (Jepson *et al.* 1994, Candolfi *et al.* 2000).

Sweet corn represents an ideal crop system for comparing potential non-target effects of transgenic Bt and conventional insecticide control. This crop is heavily treated with insecticides for lepidopteran pests, and has a shorter crop cycle than other transgenic crops. Thus, there is greater chance of ecological disruption, at least from insecticides, and less time for nontarget populations to recover from these disturbances before the end of the crop cycle. Sweet corn provides a favorable habitat for many types of natural enemies and harbors a variety of prey or host organisms, if insecticides are not applied. Copious pollen, produced during anthesis, serves as a supplemental protein source for many beneficial organisms. Because sweet corn is harvested at a premature stage, endotoxin expression is consistently high throughout the crop cycle and generally higher in certain tissues than in Bt field corn.

Field studies reported here were conducted in Maryland in 2000 and 2001 to determine the effects of Bt sweet corn on the non-target invertebrate community, with special emphasis on natural enemies.

MATERIALS AND METHODS

Study Design

Studies were duplicated each year at two research farms (Upper Marlboro and Salisbury) where the experimental conditions differed with respect to tillage systems and methods of insecticide treatments. In each experiment, plots were planted in early May and arranged in a split-plot design with four replicate blocks. Whole plot treatments included two hybrid types: (1) Bt hybrid Attribute GSS0966®, which is a yellow sugar-enhanced type for fresh market and (2) its nontransgenic isoline, Prime Plus®. Subplots of each hybrid type were either treated or untreated with lambda-cyhalothrin at the label rate. Subplots consisted of 16 rows that were 30 m long. Bt-treated subplots received one application at 100% fresh silk, which is the recommended spray regime if supplemental control is needed for high lepidopteran pressure or non-target secondary pests. Treated subplots in the nontransgenic corn received five applications starting at early silk and spaced three days apart. This spray schedule is recommended for high insect pest populations, which normally occur during mid-July when sweet corn is silking. Standard agronomic practices were used including overhead irrigation as needed and rotary mowing after harvest to shred crop residue.

Data Collection

The abundance and diversity of invertebrates representing foliage-dwelling and soil surface communities were monitored by whole plant inspections, pitfall traps, sticky cards, and litter-soil extractions throughout the growing season and in the post-harvest period. All sampling was conducted within the central third of each plot to avoid potential edge effects. Plant inspections consisted of examining 8 randomly chosen plants per plot, starting at mid-whorl and then continued weekly until harvest. All surfaces of each sampled plant were examined, including areas beneath the sheath and husk leaves and inside the ears. Surface-dwelling invertebrates were monitored with four pitfall traps per plot over 7-day intervals. Most invertebrates collected in pitfall traps were categorized to the order or family level. Carabids were assigned to genera. Four yellow sticky cards per plot were supported on cane poles at canopy level during the whorl-tassel stages and at ear level from anthesis until harvest. Exposed sticky cards were viewed under a stereomicroscope to categorize each insect to family or order. Four weeks after plots were harvested and rotary mowed, four 0.1 m^2 samples of surface litter and approximately 0.6 cm of the upper soil layer were randomly collected per plot and processed in the laboratory through Berlese funnels. The extracted organisms were grouped by family.

Statistical Analyses

Treatment and time effects on the mean abundance of each taxonomic group were tested by ANOVA. To test for treatment by time effects at the community level, principal response curve (PRC) analysis was used to distill the time-dependent, community-level effects of the treatments into a graphical form (Van den Brink and Ter Braak 1999). This multivariate method uses linear models similar to the linear model underlying regression analysis and summarizes all information on the recorded taxa simultaneously. The principal response, which is a weighted sum of the abundances of the taxa, was expressed as a canonical coefficient and reflected the behavior of the Bt or treated communities relative to the untreated nontransgenic control. A Monte-Carlo permutation method was used to test for significant departures from the control community.

RESULTS AND DISCUSSION

Diversity of Taxa and Ecological Guilds

In all experiments, over a half million organisms, representing 177 taxonomic groups and 101 recognized families, were enumerated and many more families are yet to be identified. The results presented here are based on data collected from the Upper Marlboro site in 2001 and focus primarily on the natural enemies.

In terms of diversity and abundance, approximately 78% of the invertebrates were decomposers, mainly dominated by soil-litter detritivores such as springtails (families Sminthuridae, Entomobryidae, and Isotomidae), broad mites (Parsonemidae), oribatid mites (Oribatida), psocids (Psocoptera), sowbugs (Isopoda), millipedes (Diplopoda), and various fly larvae (Diptera). Included in this ecological guild was a diverse assemblage of small beetles that fed primarily on fungal growth associated with degraded pollen and senescent plant tissue. Eight percent of the invertebrates recorded were herbivorous insects feeding on various parts of the corn plant. The most dominant taxa were aphids (Aphididae), thrips (Thripidae), leafhoppers (Cicadellidae), leafminers (Agromyiidae), flea beetles (Chrysomelidae), plant bugs (Miridae), and lepidopteran larvae. Of the higher trophic levels, 12% were foliage- and ground-dwelling predators, primarily minute pirate bugs (Anthocoridae), ladybird beetles (Coccinellidae), green lacewings (Chrysopidae), predaceous mites (Mesostigmata), ants (Formicidae), spiders (primarily Lycosidae), ground beetles (Carabidae), rove beetles (Staphylinidae), and centipedes (Geophilomorpha). Only 2% of all invertebrates counted in the study were parasitic. Most of these were Hymenoptera and Diptera captured in sticky trap as adults.

Foliage-Dwelling Community

The principal responses of the invertebrate communities recorded by visual inspection of plants are shown in Fig. 1. The control response indicated by open triangles is the untreated non-Bt community with coefficients set to zero. The Monte-Carlo test indicated a significant treatment by time effect, which was due primarily to departures from the control community in the insecticide-treated plots. Changes in community structure coincided with insecticide applications and were greater in non-Bt plots sprayed five times compared with Bt plots sprayed once. The response curve for the untreated Bt plots fluctuates around the zero line of the control indicating that no significant changes in community structure occurred. The PRC analysis provided weighted scores for each taxonomic group, listed in Fig. 1. The scores represent the relative contributions to the principal response. Taxa with high positive weights behaved the same as the patterns indicated by the curves, whereas taxa with near zero weight (between -0.5 to 0.5) showed no change or an unrelated response. The predator groups that were most significantly reduced in abundance by the insecticide application included *Orius* flower bugs, coccinellids, lacewing eggs, spiders, and soldier beetles. The treatment by time responses are also shown in Fig. 2, which shows the average densities per plant of the major predator groups. There were no significant differences in densities and population trends of predators in the untreated Bt and non-Bt plots. Overall predator communities in the treated plots were reduced by 55% after one insecticide application in the Bt plots, and by 76% as a result of five applications in the non-Bt plots.

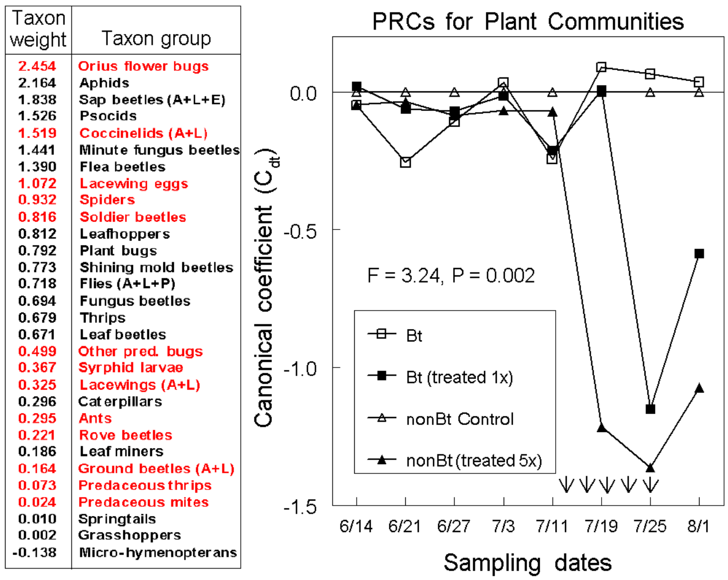

Figure 1. Principal response curve (PRC) and weights for taxonomic groups of foliage-dwelling invertebrates recorded by visual inspections of individual plants.

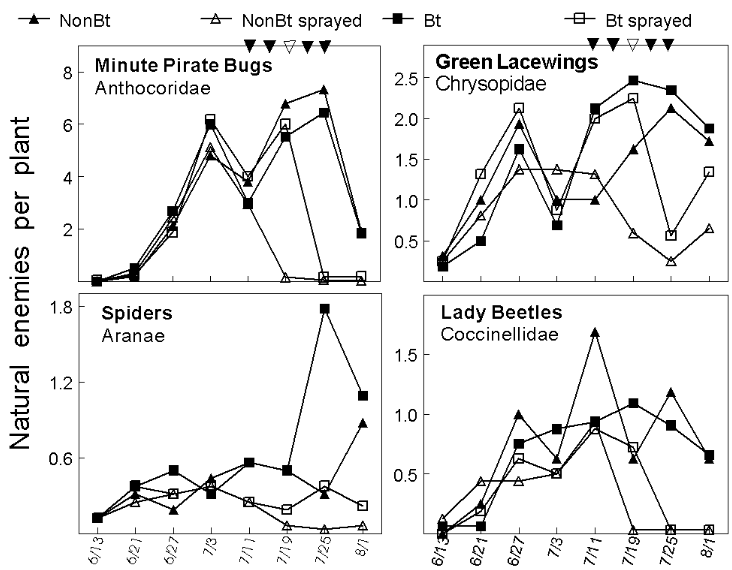

Figure 2. Effects of Bt transgenic and conventional insecticide regimes on the population densities of the major natural enemy groups as measured by visual plant inspections. Triangular markers at top margins indicate timing of insecticide applications in non-Bt plots (open markers indicate application in Bt plots).

Aerial Community

Of the flying invertebrates captured by sticky cards, 15% were predators or parasitoids, primarily parasitic hymenopterans and minute pirate bugs. Of the parasitic wasps, scelionids, mymarids, braconids, and trichogrammatids comprised 82% of the total recorded. The PRC analysis (Fig. 3) showed no differences between aerial communities in the Bt and non-Bt plots but significant departures from the control community when insecticides are applied. Reductions in the weighed abundances of taxa were greatest in non-Bt plots that received five applications of insecticides. Of the natural enemies with high positive weights, predaceous bugs, coccinellids, and scelionid parasitoids were the most adversely affected by the insecticide. Taxa with high negative weights behaved in the opposite way to the patterns indicated by the curves; thus, thrips, mites, and aphids exhibited an increase in the insecticide-treated plots. Resurgence of these organisms is a common response after treatments of pyrethroid insecticides. The same response trends are shown in Fig. 4, summarizing the population densities of parasitic wasps, minute pirate bugs, predaceous/parasitic flies, and coccinellids. There were no significant differences in the population trends over time for the Bt and non-Bt natural enemy communities. However, the natural enemies in insecticide-treated plots were reduced by 21% and 33% following one and five applications, respectively. Populations of parasitic hymenopterans seemed to be less sensitive to insecticides than expected, and this was apparently due to high rates of recruitment replacing adult parasitoids.

Surface-Dwelling Community

The community of natural enemies measured by pitfall traps consisted primarily of ants, spiders (>90% wolf spiders), rove beetles, predaceous mites, and ground beetles. These predator groups comprised 24% of the total captures. Staphylinids were the most abundant coleopteran group, averaging 13.6 beetles per trap per week. Of the carabids, which averaged 3.1 beetles per trap, the genera *Harpalus*, *Stenolophus*, *Scarites*, *Amara*, *Pterostichus*, and *Chlaenius* were the most predominate of the 18 genera recorded. Most of the ground beetles captured were predaceous on other insects, except for the genera *Stenolophus* and *Amara*, which contain many seed feeders. The principal response curves (Fig. 5) for the ground surface communities indicated a significant treatment by time effect and clearly showed that the diversity and composition of the treated Bt and non-Bt communities changed after insecticides were applied. As indicated by the higher positive weights, rove beetles, spiders, and coccinellids were most significantly reduced by insecticide applications. Ants and ground beetles were the least affected. Coefficients for the Bt community deviated slightly around the control line, indicating no significant departures from the non-Bt community structure. These responses are consistent with the population trends of individual predator groups depicted in Fig. 6. The overall density of the natural enemy complex on the ground surface was highest at the mid whorl stage and then declined as the plant canopy closed in. Population trends of individual predator groups were not significantly different between the untreated Bt and non-Bt communities. However, overall predator populations in the insecticide-treated plots were reduced by 48 to 70% with one and five applications, respectively. Consistent with the PRC weighing scores, rove beetles and spiders were the most disrupted, while ants and carabids were the least affected.

Taxon weight	Taxon group
2.83	Predaceous bugs
2.24	Lady beetles
2.16	Minute fungus beetles
2.10	Shining mold beetles
1.91	Grass flies
1.87	Picture-winged flies
1.69	Mirids/other plant bugs
1.24	Scelionids
1.19	Leafhoppers
1.17	Flea beetles
1.04	Midges
0.85	Flesh flies
0.83	Humpbacked flies
0.77	Braconids
0.75	Tachinids
0.63	Pomace flies
0.60	Chalcid egg parasites
0.56	Rove beetles
-0.57	Springtails
-1.03	Aphids
-1.14	Mites
-2.09	Thrips

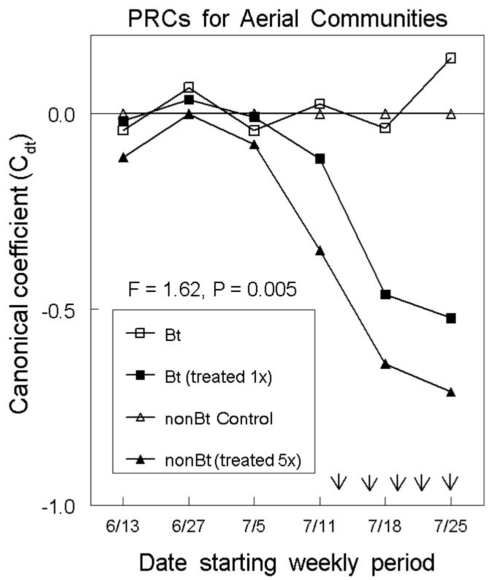

Figure 3. Principal response curve (PRC) and weights for taxonomic groups of foliage-dwelling invertebrates recorded by sticky cards.

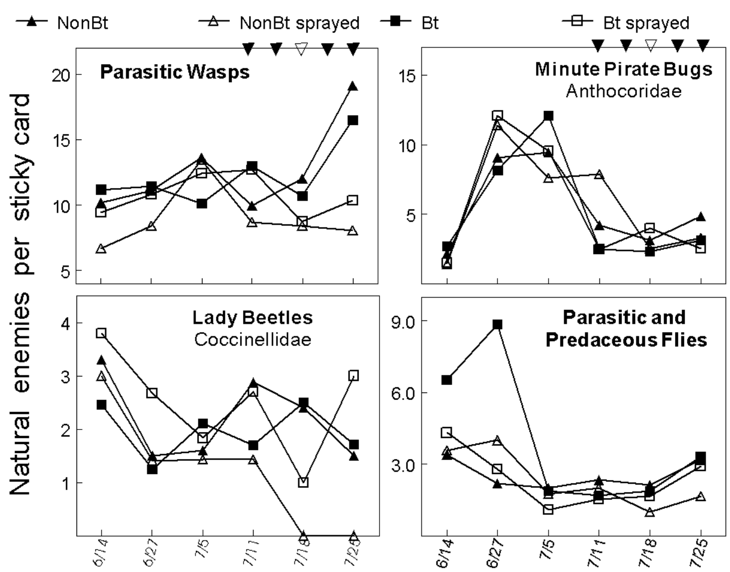

Figure 4. Effects of Bt transgenic and conventional insecticide regimes on the population densities of the major natural enemy groups as measured by sticky cards. Triangular markers at top margins indicate timing of insecticide applications in non-Bt plots (open markers indicate application in Bt plots).

Taxon weight	Taxon group
3.66	Rove beetles
2.58	Crickets
1.84	Spiders
1.32	Lady beetles
0.49	Leaf/planthoppers
0.41	Flies
0.24	Aphids
0.17	Lacewing larvae
0.15	Misc. beetle larvae
0.11	Psocids
0.10	Soldier beetles
0.10	Click beetles
0.08	Predaceous bugs
-0.05	Ants
-0.17	Millipedes
-0.18	Misc. beetles
-0.22	Slugs
-0.34	Thrips
-0.34	Fungus beetles
-0.53	Ground beetles
-0.56	Predaceous mites
-0.58	Centipedes
-0.58	Flea beetles
-0.61	Mirids/plant bugs
-0.78	Oribatid mites
-0.83	Springtails

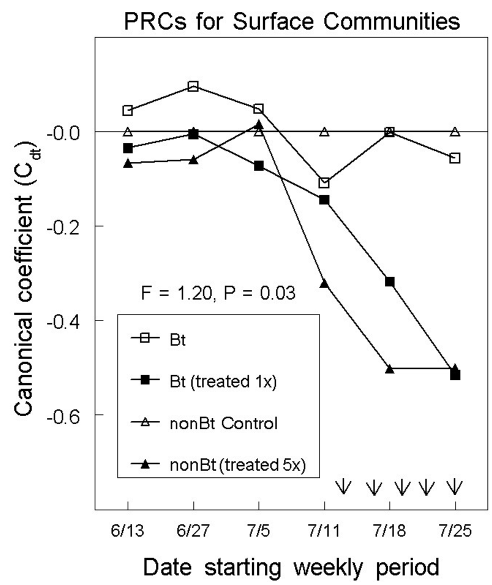

Figure 5. Principal response curve (PRC) and weights for taxonomic groups of surface-dwelling invertebrates recorded by pitfall traps.

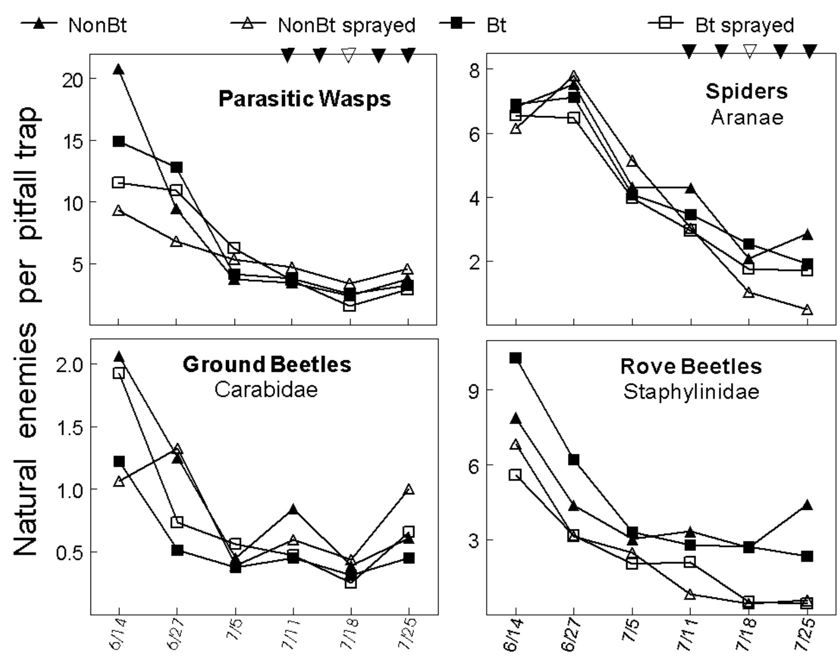

Figure 6. Effects of Bt transgenic and conventional insecticide regimes on the population densities of the major natural enemy groups as measured by pitfall traps. Triangular markers at top margins indicate timing of insecticide applications in non-Bt plots (open markers indicate application in Bt plots).

Soil-litter Community

The invertebrate community in the litter four weeks after harvest was the most diverse, consisting of more than 60 taxonomic groups and predominately made up of saprophytes (springtails, mites, various beetle adults and larvae, and fly larvae) and predators (rove beetles, ants, carabids, spiders). The PCR analysis indicated no significant departures from the non-Bt control community. However, as shown by the bar graphs in Fig. 7, densities of individual predator groups per m^2 of surface litter were significantly different among the four treatment combinations. Litter populations of all predators were consistently higher in the Bt plots and generally lower in the insecticide-treated plots. The higher predator populations in Bt litter were not intuitive because surface-dwelling communities were essentially the same in the untreated Bt and non-Bt plots during the growing cycle of the crop. It is unclear why these differences occurred.

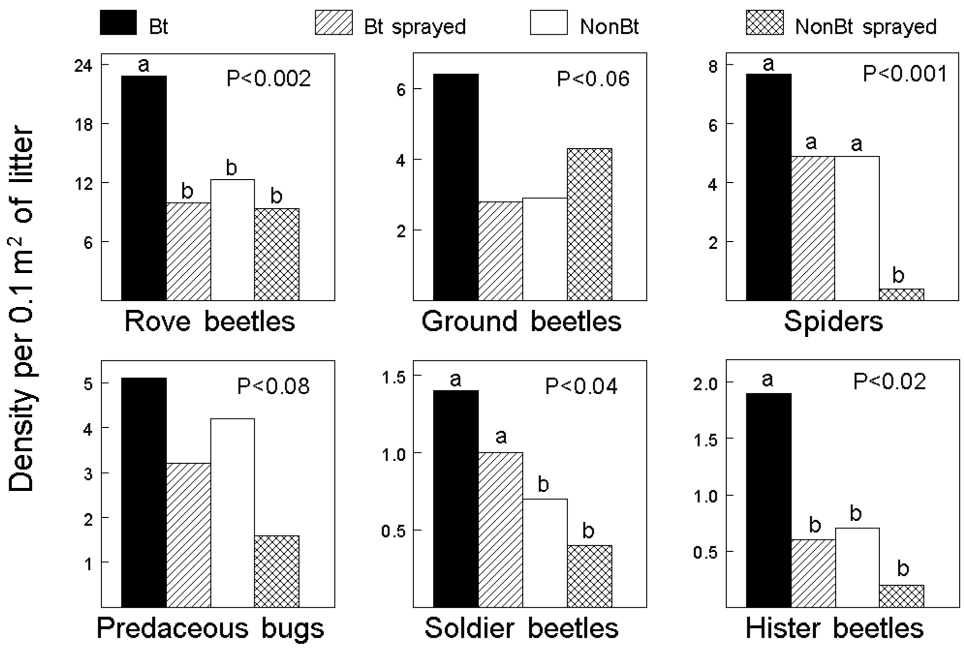

Figure 7. Average densities of the major predator groups found in soil-litter samples at four weeks after harvest in the four treatment combinations. P values indicate significance of the main treatment effect. Mean bars within each predator group with the same letter are not significantly different at the 5% probability level.

CONCLUSIONS

As expected, Cry1Ab expression significantly reduced the densities and damage caused by European corn borer and corn earworm. The absence of lepidopteran larvae had little impact on the natural enemy complex because they represented a very small portion of the available host and prey items. Only a few host-specific parasitoids of European corn borer were affected.

Populations of sap beetles were significantly lower in Bt sweet corn. These beetles are attracted to the plant and ear injury produced by lepidopteran pests, which was lacking in the Bt plots. There was no evidence of any adverse effects on other nontarget herbivores and decomposers in the untreated Bt plots. Furthermore, the Bt hybrid had no significant negative effects on the abundance and diversity of natural enemies enumerated at the family level by the various sampling methods during the crop cycle. However, densities of predators in the Bt litter four weeks after harvest were significantly higher. As expected, the insecticide lambda-cyhalothrin had broad negative impacts on pest species and many nontarget invertebrates. One insecticide application in the Bt plots reduced communities of natural enemies by 21 to 48%. Five applications in the non-Bt plots reduced natural enemy communities by 33 to 70%. Non-target communities affected by one application in the Bt plots exhibited some recovery, but invertebrate communities exposed to five applications showed no trends toward recovery during the crop cycle or post-harvest in the crop refuge.

In summary, results of this study lend strong support to the findings of other published reports (Pilcher *et al.* 1997, Orr and Landis 1997, Lozzia 1999, Zwahlen *et al.* 2000) that there are no unexpected tritrophic effects from transgenic lepidopteran-resistant corn on nontarget organisms. As indicated above, some changes in certain taxa did occur, but these changes were indirectly linked to plant-mediated factors and the absence of feeding injury by target pest species. Communities of nontarget invertebrates in the insecticide-sprayed plots displayed significant structural changes that resulted in resurgence in certain species. Whether these ecological disturbances carry over to the following season or have landscape-scale consequences remains unknown. However, it is clear that the community-level impacts of insecticide control on natural enemies and other non-targets are far greater than any effect of Bt transgenic control.

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