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# Effects of Insecticide-Treated and Lepidopteran-Active *Bt* Transgenic Sweet Corn on the Abundance and Diversity of Arthropods

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**ABSTRACT** A field study was conducted over 2 yr to determine the effects of transgenic sweet corn containing a gene from the bacterium *Bacillus thuringiensis* (*Bt*) on the diversity and abundance of nontarget arthropods. The *Bt* hybrid (expressing Cry1Ab endotoxin for lepidopteran control) was compared with near-isogenic non-*Bt* and *Bt* hybrids treated with a foliar insecticide and with a near-isogenic non-*Bt* hybrid without insecticides. Plant inspections, sticky cards, and pitfall traps were used to sample a total of 573,672 arthropods, representing 128 taxonomic groups in 95 families and 18 orders. Overall biodiversity and community-level responses were not significantly affected by the transgenic hybrid. The *Bt* hybrid also had no significant adverse effects on population densities of specific nontarget herbivores, decomposers, and natural enemies enumerated at the family level during the crop cycle. As expected, the insecticide lambda-cyhalothrin had broad negative impacts on the abundance of many nontarget arthropods. One insecticide application in the *Bt* plots reduced the overall abundance of the natural enemy community by 21–48%. Five applications in the non-*Bt* plots reduced natural enemy communities by 33–70%. Nontarget communities affected in the insecticide-treated *Bt* plots exhibited some recovery, but communities exposed to five applications showed no trends toward recovery during the crop cycle. This study clearly showed that the nontarget effects of *Bt* transgenic sweet corn on natural enemies and other arthropods were minimal and far less than the community-level disruptions caused by lambda-cyhalothrin.

**KEY WORDS** *Bt* sweet corn, nontarget, Cry1Ab, principal response curves

Sweet corn containing a gene derived from the bacterium *Bacillus thuringiensis* (*Bt*) was introduced commercially in 1998, as ATTRIBUTE insect-protected hybrids by Novartis Seeds (now Syngenta Seeds, Golden Valley, MN). The original transformation introduced a *cry1Ab* gene and a *pat* marker gene into field corn. The resulting transgenic Event Bt11 field corn was crossed by traditional breeding with sweet corn. Constitutive expression of the Cry1Ab endotoxin provides exceptional control, preventing virtually 100% of the damage normally caused by European corn borer (*Ostrinia nubilalis* Hübner) and providing >90% control of the corn earworm (*Helicoverpa zea* Boddie) and fall armyworm (*Spodoptera frugiperda* Smith) when normal insect populations occur. Use of *Bt* sweet corn can potentially reduce the number of insecticide applications by 70–90% (G.P.D., unpublished data; Musser and Shelton 2003).

Like any insect control technology, transgenic expression of an insecticidal *Bt* protein may present a risk to the nontarget arthropod community. Tiered laboratory tests indicated no adverse effects on a suite of individual nontarget organisms, as did studies con-

ducted to assess community-level effects (reviewed by Romeis et al. 2006). *Bt* sweet corn can potentially interact both directly and indirectly with nontarget organisms at different trophic levels (Schuler et al. 1999). A reduction in host or prey populations, indirect contact with endotoxin 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. Reductions in insecticide use resulting from planting *Bt* sweet corn could be beneficial to natural enemies and reduce overall environmental impacts (Betz et al. 2000). Conservation of predators and parasitoids in *Bt* fields may aid in controlling secondary nontarget pests. Thus, an assessment of the ecological risks of *Bt* sweet corn on nontarget organisms should include a comparison with the risks of conventional control methods. Furthermore, risks should be assessed at a spatial scale large enough to represent typical agronomic conditions and involve a broad taxonomic range of organisms at the community level (Jepson et al. 1994, Candolfi et al. 2000).

Reported here is a field study to determine the community-level effects of *Bt* sweet corn on the abundance and diversity of nontarget arthropods, with emphasis on natural enemies. Sweet corn represents an ideal crop system for comparing the ecological risks of transgenic *Bt* and conventional insecticide control.

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The crop typically is heavily treated with insecticides for lepidopteran pests and has a shorter crop cycle than other transgenic crops. 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. Thus, there is a 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 also provides a favorable habitat for many natural enemies and harbors a variety of prey and host organisms if insecticides are not applied. Copious pollen, produced during anthesis, serves as a supplemental protein source for many beneficial organisms.

## Materials and Methods

### Plot Design and Treatments

Replicated field plot experiments were conducted over two growing seasons (2000 and 2001) and duplicated at two locations within each year. The sweet corn hybrid 'Attribute GSS0966' and its nontransgenic isogenic counterpart ('Prime Plus'; Syngenta Seeds) were planted at the University of Maryland Research and Education facilities at Upper Marlboro and Salisbury, MD. Both plantings were irrigated and grown according to recommended commercial practices but differed with respect to the tillage system. At Upper Marlboro, plots were planted into no-till fields consisting of old corn stubble with a killed rye cover crop. Each block was 16 rows, spaced 0.75 m apart and 39 m long. Sweet corn plots at Salisbury were conventionally tilled and consisted of 24 rows, spaced 0.75 m apart and 30 m long. At both locations, a 10-m noncropped buffer separated each block.

In both experiments, plots were laid out in a 2 by 2 split-plot design with four replicate blocks. The two hybrids (*Bt* and non-*Bt*) were arranged as whole plots, whereas the subplots were either untreated or treated with lambda-cyhalothrin (Warrior 1E insecticide; Syngenta Crop Protection, Greensboro, NC) at a rate of 237 ml/ha. Treatments were applied at Upper Marlboro with an airblast sprayer delivering 536 liters/ha of spray volume and at Salisbury with a high clearance drop nozzle sprayer delivering 475 liters/ha of spray volume. Treated isogenic nontransgenic plots received five sprays starting at early silking and repeated every 3 d. This represented a typical schedule for control of ear-invading insects in late-season plantings in Maryland. Treated *Bt* plots received one insecticide application at 100% fresh silk, which coincided with the third application in the nontransgenic plots. This supplemental treatment is often recommended to provide protection against secondary nontarget pests or high populations of corn earworm. All plots were rotary mowed 1 wk after harvest of fresh-market ears (18–21 d after the onset of anthesis).

### Data Collection

Whole plants randomly selected from the central rows of each plot were carefully examined to enumerate plant-dwelling arthropod taxa. Sampling started during the late-whorl stage and continued weekly until harvest. At each time, four and eight plants per plot were sampled in 2000 and 2001, respectively. All arthropods found on the tassel, leaf and stalk surfaces, silks, and inside the ear were identified and recorded to order or family level. Yellow sticky cards were used to measure relative numbers of aerially active arthropods in the plant canopy. This technique measured many mobile insects that were not recorded by other sampling methods. Four sticky cards (7.5 by 12.5 cm; Olson Products, Medina, OH) were placed within the center four to five rows of each plot. Cards were positioned at canopy level through the tassel stages and at ear level during anthesis until harvest. Cards were exposed for 7-d periods four times during July in 2000 and six times in 2001 from mid-June through July at both locations. After exposure, cards were placed in clear plastic resealable bags and brought to the laboratory where captured arthropods were identified under a stereo microscope and recorded to order or family level.

Surface-dwelling arthropods were monitored over weekly intervals with pitfall traps, using the same design and placement described by Dively (2005). Four pitfall traps were placed in each plot in close proximity to the yellow sticky traps. At Upper Marlboro, pitfall samples were collected eight times during June through August 2000 and six times from mid-June through July in 2001. At Salisbury, six samples were collected during July through mid-August in 2000, and four samples were collected during July in 2001. Captured arthropods were vacuum filtered over a fine organdy screen to remove the ethylene glycol. Filtered organisms were stored in 70% ethanol and later identified to order or family levels. Carabids were identified to the genus level, and voucher specimens were deposited at the University of Maryland.

### Statistical Analyses

The overall community composition of arthropods sampled by each method was characterized by computing the frequency of occurrence and mean density per sample unit for each location and year. For univariate analysis, data were tested for normality using the Shapiro-Wilk's *W* test and for homogeneity of variance by looking for nonrandom patterns in residual plots. Appropriate transformations were performed and/or variances were grouped before analysis to correct for skewness and heterogeneous variances. For density measurements of individual taxa, data over years were treated as repeated measurements because each plot was exposed to the same treatment each year. To increase statistical power, data from both years for each location were averaged by matching sampling dates to corresponding weekly intervals before and after the first insecticide appli-

cation at early silking. This effectively synchronized the data sets on the basis of plant phenology. However, groupings of sticky card and pitfall data relative to the first application were not possible in all cases because the 7-d sampling periods did not always start at the time of application. A mixed model analysis of variance (ANOVA) (SAS Institute 1997) was used to test for treatment and interaction effects on selected arthropods or combined taxonomic groups for each location. Treatment and weekly sampling time were considered fixed factors, whereas replicate was treated as a random block effect. The repeated measures option with the most appropriate covariance structure was used to correct for temporally correlated data. Significant effect means were separated using Tukey's adjustment for pairwise comparisons ( $P < 0.05$ ).

Because of the complexity of the data sets, several multivariate approaches also were used to simultaneously examine and summarize all taxa recorded by each sampling method, thus allowing for an evaluation of treatment effects on the sampled community as a whole. The Shannon-Wiener (S-W) diversity index (Shannon and Weaver 1949) was used to compare the community diversity of the arthropod fauna. For each sampling method, indices for individual samples were computed at each sampling time. Mixed model ANOVA was used to test for time, treatment, and interaction effects.

The principal response curve method (PRC) was used to reduce the time-dependent, community-level effects of the treatments into a graphical form. This method has been used recently to analyze responses of nontarget communities to *Bt* crops (Candolfi et al. 2003, Dively 2005, Naranjo 2005, Prasifka et al. 2005, Torres and Ruberson 2005, Whitehouse et al. 2005), and its advantages are explained by Van den Brink and Ter Braak (1999). A Windows-based program (CANOCO version 4.51, Biometris; Plant Research International, Wageningen, The Netherlands) generated the canonical coefficients for the *Bt*, insecticide-treated *Bt*, and insecticide-treated isogenic treatments plotted over time. The line graph produced by these coefficients represented the principal response and reflected the weighted sum of the abundances of all taxa. The arthropod community in the untreated, isogenic plots was designated the undisturbed control community, and its response was represented as a horizontal time trajectory set at zero. This allowed graphical comparisons of the behavior of each treated or *Bt* community as departures from the control community. The significance of the principal responses was tested by Monte-Carlo permutations. The null hypothesis was that the canonical coefficients equaled zero for all times and treatments or were not different from the horizontal control line. A shuffling sequence for generating permutations of the original data set was designed to remove blocking and repeated measures effects as sources of error. The permutation procedure generated 1,000 new sets of data that were equally likely under the null hypothesis, while keeping the treatment and time structure of the data fixed. The significance level was calculated by the proportion of

$F$  values greater than or equal to the  $F$  value based on the original data set.

The multivariate analysis also generated taxon-specific weights, which reflected the relative contribution of each taxon to the principal response. These weights were used to identify which taxa were most affected by the treatments. Populations of arthropods with high positive weights were inferred to likely follow the pattern shown by the principal response, whereas taxa with greater negative weights were inferred to show the opposite pattern. Taxa with weights near zero ( $-0.5$  to  $0.5$ ) were arbitrarily considered to show either no response or one that was unrelated to the pattern shown by the principal response. Treatment effects on individual or pooled taxa that contributed significantly to the principle response were analyzed further by mixed model ANOVA.

## Results

### Diversity of Taxonomic Groups

Summed over years and locations, 573,672 adult and immature arthropods were identified and counted, representing 128 taxonomic groups in 95 families and 18 orders. The diversity and abundance of sampled communities were grouped by ecological function into decomposers, herbivores, parasitoids, and predators. Decomposers were the most abundant group, comprising 39% of the arthropods recorded. Most of these organisms were found in surface litter residue or on the plant associated with degraded pollen, open wounds caused by caterpillar injury, or senescent plant tissue. The most abundant decomposers included broad mites (Tarsonemidae), oribatid mites (Oribatida), springtails (Sminthuridae, Entomobryidae, and Isotomidae), psocids (Psocoptera), flies (Diptera), and fungivorous beetles (Nitidulidae, Corylophidae, Phalacridae, Cryptophagidae, Oedermeridae, Anthicidae, Mycetophagidae, and Lathridiidae).

Plant- and surface-dwelling predators were the second most abundant trophic group comprising 32% of all arthropods. Predominant taxa included insidious flower bugs (Anthocoridae), lady beetles (Coccinellidae), green lacewings (Chrysopidae), predaceous mites (Mesostigmata), ants (Formicidae), spiders (primarily Lycosidae), ground beetles (Carabidae), rove beetles (Staphylinidae), and centipedes (Chilopoda). Twenty-four percent of the arthropods were herbivores, which were mainly recorded in the plant samples. Of these plant feeders, aphids (Aphididae), thrips (Thripidae), leafhoppers (Cicadellidae), leafminers (Agromyzidae), flea beetles (Chrysomelidae), and plant bugs (Miridae) were the most abundant. Only 5% of all arthropods identified in the study were parasitoids, and these insects were largely collected on sticky traps. The parasitoids were mostly hymenopterans (Scelionidae, Mymaridae, Trichogrammatidae, Pteromalidae, Charipidae, Encyrtidae, Aphelinidae, Ceraphronidae, Eulophidae, Braconidae, Ichneu-

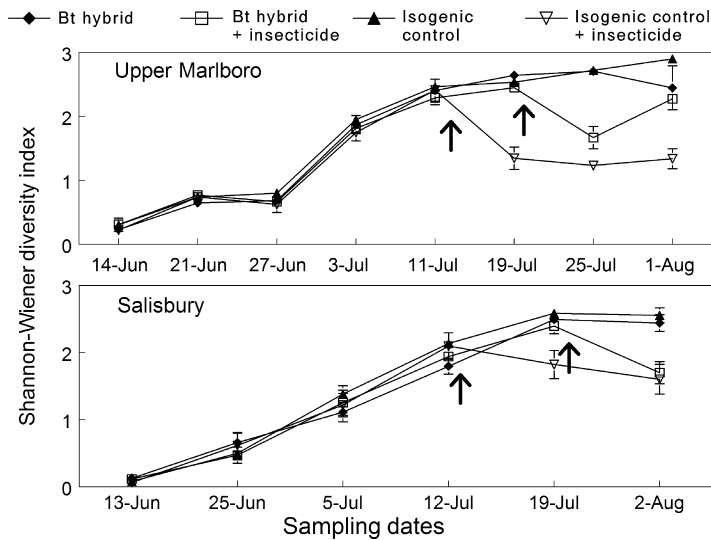


Fig. 1. Mean Shannon-Wiener diversity indices of arthropod communities recorded by plant inspections in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD, 2001. First arrow indicates first insecticide application in the treated isogenic plots, whereas second arrow indicates the single application in the treated *Bt* plots.

monidae, Chrysididae, Proctotrupidae, Pompilidae) and some dipterans (Tachinidae).

**Plant-Dwelling Community**

**Diversity.** A steady increase in the number and diversity of plant-dwelling arthropods occurred as plant biomass and structural diversity provided more habitat niches and resources. Diversity changed from simple communities of a few dominant taxa during June to more diverse communities of 25–30 taxa during July. There was a significant treatment-by-time effect for the S-W diversity indices at Upper Marlboro (2001:  $F_{(21,96)} = 9.74, P < 0.001$ ) and at Salisbury (2001:  $F_{(15,72)} = 3.6, P = 0.001$ ; Fig. 1). The number of taxa found on plants was reduced by 63 and 30% in the insecticide-treated non-*Bt* and treated *Bt* plots, respectively, but differences in the diversity index between *Bt* and nontransgenic control plots were not significant ( $P > 0.13$ ). Moreover, the diversity in *Bt* communities exposed to the single insecticide application showed evidence of recovery, whereas the non-*Bt* communities exposed to five applications continued to decline in most cases. The overall number of plant-dwelling taxa recorded in the no-till plots at Upper Marlboro was 22% higher than the number of taxa recorded in the conventional plots at Salisbury.

**Response Curves.** PRC responses of the plant-dwelling communities are shown in Fig. 2 with the response of the non-*Bt* control community indicated by the horizontal line set at zero. The first ordination axis explained 65 and 60% of the variation caused by treatment effects at the Upper Marlboro and Salisbury sites, respectively. This axis accounted for significantly more variation than the other axes, thus it was used to construct response curves. The Monte-Carlo test indicated a significant treatment effect at Upper Marl-

boro ( $F = 34.9, P = 0.001$ ) and Salisbury ( $F = 18.5, P = 0.001$ ), which was clearly caused by negative departures of the insecticide-treated communities from the control community. Changes in community structure coincided with insecticide applications and were greatest in the non-*Bt* plots receiving five insecticide applications. Responses of the untreated *Bt* community around the zero line were not statistically different from the control community ( $P > 0.08$ ). Seventeen taxa had positive weights  $>0.5$ , indicating that their abundance was reduced by the insecticide treatments (Fig. 2). The higher the positive weight, the greater the negative effect on the indicated taxonomic group. The PRC analysis also indicated that no arthropod group was positively affected by the treatments.

**Effects on Specific Taxa.** Peak densities of thrips occurred 2–3 wk before silking and averaged 36.6 and 16.8 per plant at Upper Marlboro and Salisbury, respectively. Population levels were not different among treatments and naturally declined before the insecticide treatments. Pooled over locations, aphid densities were reduced by 79 and 30% in the non-*Bt* and *Bt* insecticide-treated plots, respectively. However, because of high variability, these differences were not statistically significant. Insecticide applications reduced chrysomelids (flea beetles), cicadellids, and mirids, grouped together, by 55 and 84% in the *Bt* and non-*Bt* treated plots, respectively, but treatment-by-time effects on these herbivores were not significant in the untreated *Bt* plots (Upper Marlboro:  $F_{(18,72)} = 3.17, P < 0.001$ ; Salisbury:  $F_{(12,48)} = 3.56, P < 0.001$ ).

Nitidulids and other fungivorous beetles (mainly Corylophidae and Phalacridae) were the predominant decomposers found on plants. This trophic group was significantly reduced by 22–34% in the *Bt*-treated plots and by 76–83% in the non-*Bt*-treated plots (Upper Marlboro:  $F_{(18,72)} = 7.84, P < 0.001$ ; Salisbury:

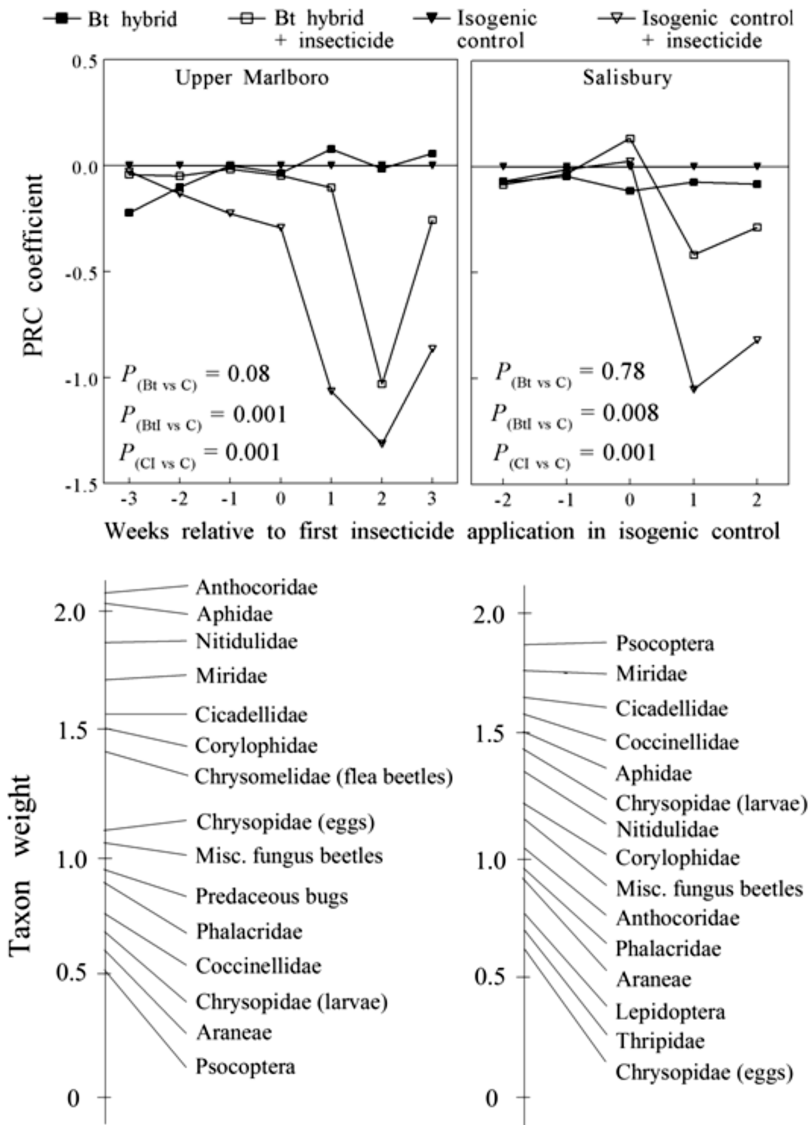


Fig. 2. PRCs and taxon weights of plant-dwelling arthropod communities exposed to *Bt* and insecticide-treated sweet corn compared with the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxa with weights between  $-0.5$  and  $0.5$  are not shown.

$F_{(12,48)} = 3.61, P < 0.001$ ; Fig. 3). Treatment-by-time differences were not significant between *Bt* and non-*Bt* control treatments, although most fungivores were numerically less abundant in *Bt* plots at the end of the crop cycle.

Densities of plant-dwelling predators, primarily chrysopids, anthocorids, coccinellids, and spiders, increased during anthesis as pollen and other food sources became available. The treatment-by-time interaction effect was significant at both locations for chrysopids (Fig. 4; Upper Marlboro:  $F_{(18,72)} = 3.11, P < 0.001$ ; Salisbury:  $F_{(12,48)} = 4.19, P < 0.001$ ); anthocorids (Fig. 5; Upper Marlboro:  $F_{(18,72)} = 21.4, P < 0.001$ ; Salisbury:  $F_{(12,48)} = 1.19, P = 0.03$ ); and coccinellids (Fig. 6; Upper Marlboro:  $F_{(18,72)} = 6.02, P <$

$0.001$ ; Salisbury:  $F_{(12,48)} = 2.09, P = 0.036$ ). These major predators were all adversely affected by the insecticide treatments, averaging 27 and 58% less overall in the *Bt*- and non-*Bt*-treated plots, respectively. Spiders also were noticeably affected by the insecticide but treatment differences were only significant at Upper Marlboro ( $F_{(3,12)} = 8.01, P < 0.003$ ). Population levels of all plant-dwelling predators did not differ statistically in the *Bt* plots compared with those in the non-*Bt* control.

**Aerial Community**

**Diversity.** Of >80 taxa captured by sticky cards, herbivores were the most abundant (50% of total),

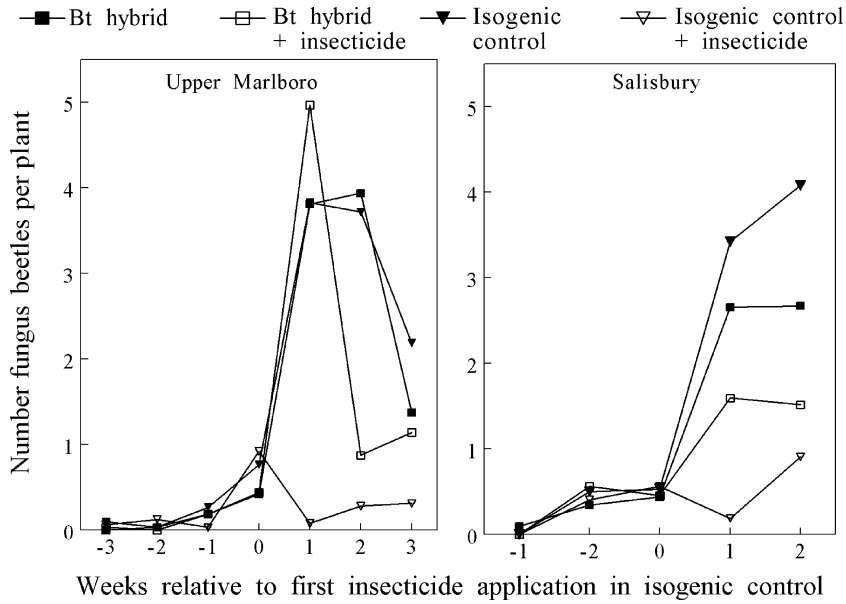


Fig. 3. Mean number of fungus beetles (primarily Nitidulidae, Corylophidae, and Phalacridae) recorded by plant inspections in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

followed by decomposers (31%), parasitoids (13%), and predators (7%). Taxa richness and diversity of aerial arthropods were significantly affected by the insecticide treatments, but differences were less than and not as consistent as those recorded by plant inspections. At Upper Marlboro, there was a significant treatment-by-time interaction effect on diversity (2000:  $F_{(9,48)} = 2.01, P = 0.06$ ; 2001:  $F_{(15,72)} = 2.65, P =$

0.003). This effect was not significant at Salisbury, except for the treatment-by-time interaction for the S-W indices in 2000 ( $F_{(9,48)} = 2.25, P = 0.034$ ). Generally, taxa diversity was lowest on the last sampling dates in non-*Bt* plots that received five insecticide applications. Additionally, there was no consistent trend of any reduction in community diversity in the *Bt* plots compared with the non-*Bt* control.

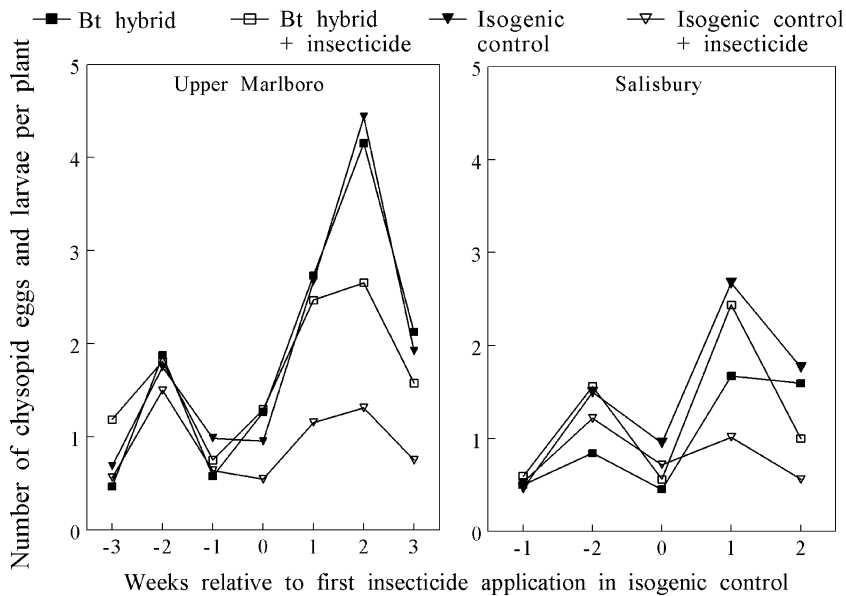


Fig. 4. Mean number of chrysopids (eggs, larvae, and adults) recorded by plant inspections in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

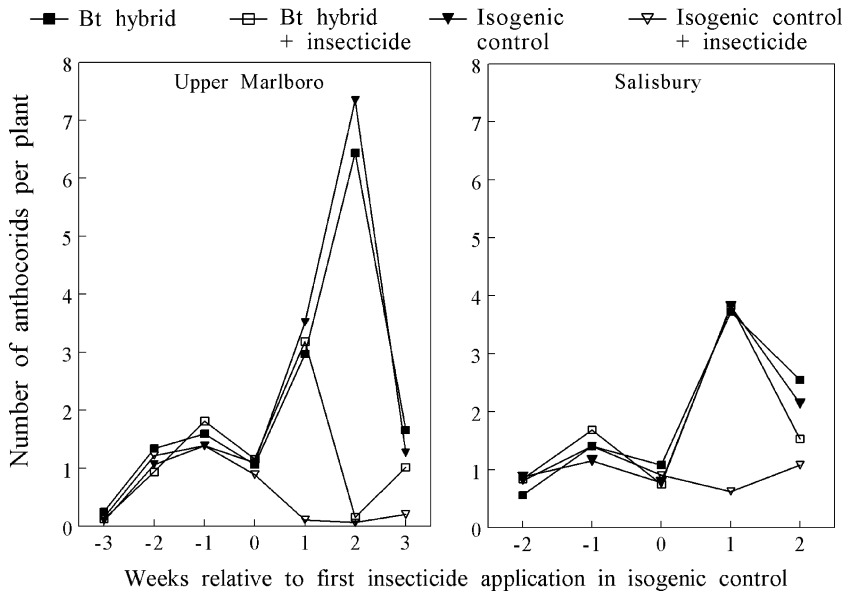


Fig. 5. Mean number of anthocorids recorded by plant inspections in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

**Response Curves.** The PRC analysis showed no differences between aerial communities in the *Bt* and non-*Bt* control plots, but significant negative departures from the control community when insecticides were applied at both locations (Fig. 7). The majority of the variation caused by the treatment effect was explained by the first ordination axis (43.9% for Upper Marlboro; 34% for Salisbury). The treatment effect was

significant as indicated by negative changes in the weighed abundances of taxa over time relative to the control community (Upper Marlboro:  $F = 8.8$ ;  $P = 0.001$ ; Salisbury:  $F = 5.6$ ;  $P = 0.001$ ). Reductions were greatest in non-*Bt* plots receiving five insecticide treatments compared with *Bt* plots treated once. The analysis indicated that 24 taxa with positive weights were likely reduced in the insecticide-treated plots. Of

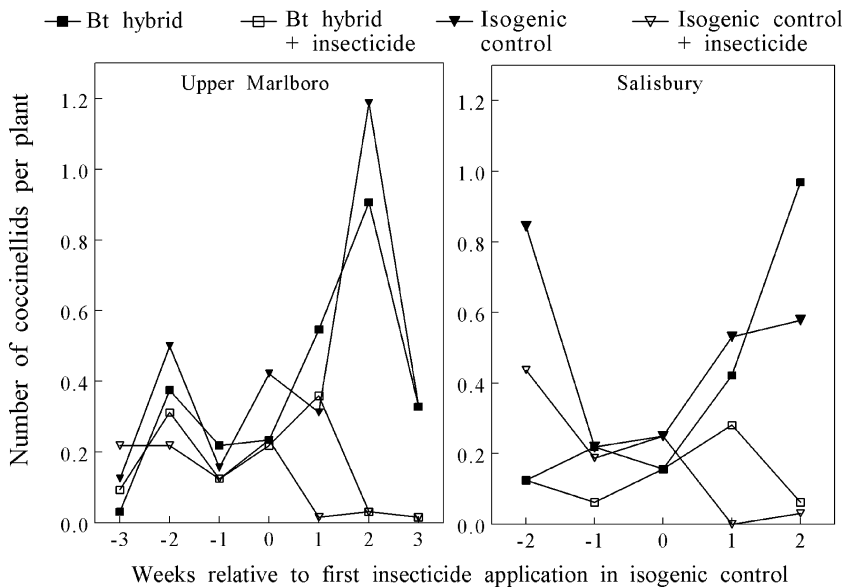


Fig. 6. Mean number of coccinellids recorded by plant inspections in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).



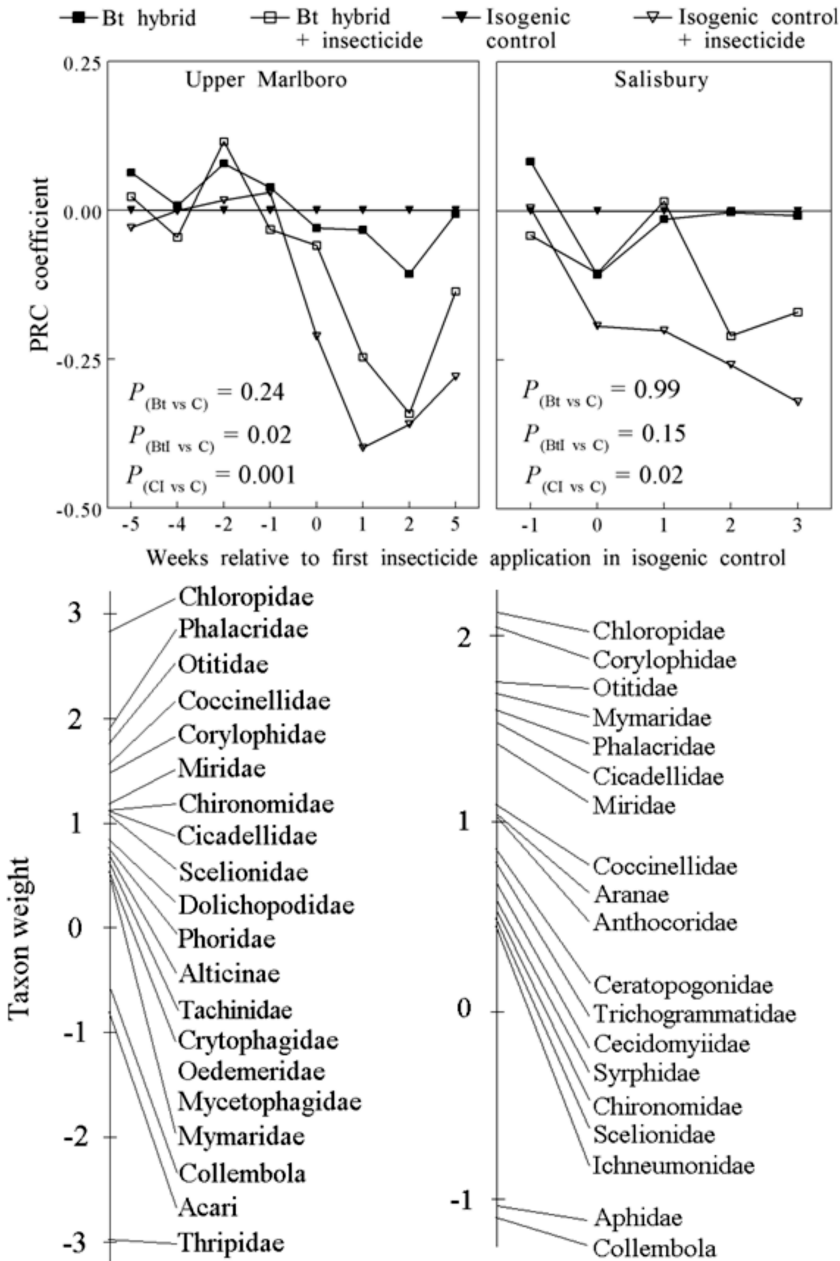


Fig. 7. PRCs and taxon weights of aerial arthropod communities exposed to *Bt* and insecticide-treated sweet corn compared with the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxa with weights between  $-0.5$  and  $0.5$  are not shown.

these taxa, dipterans (Chloropidae, Otitidae), fungus beetles (Phalacridae, Corylophidae), plant bugs (Miridae), leafhoppers (Cicadellidae), coccinellids, and parasitic wasps (Mymaridae, Scelionidae) were the most adversely affected. Negative weights of collembolans, mites, aphids, and thrips contributed in the opposite direction; thus, they were positively affected by the insecticide treatments.

**Effects on Specific Taxa.** Sticky traps provided the most accurate assessment of parasitic Hymenoptera,

which were too small and mobile to count directly on plants. Egg and larval parasitoids in the families Scelionidae, Mymaridae, Trichogrammatidae, Pteromalidae, Encyrtidae, Braconidae, Ceraphronidae, and Aphelinidae comprised 91% of all hymenopterans recorded and averaged 11.4 per sticky card. Because the PRC weights (Fig. 7) indicated that parasitoids responded similarly to the treatment effects, densities of all families were pooled as a functional group. The overall means plotted in Fig. 8 show a significant main

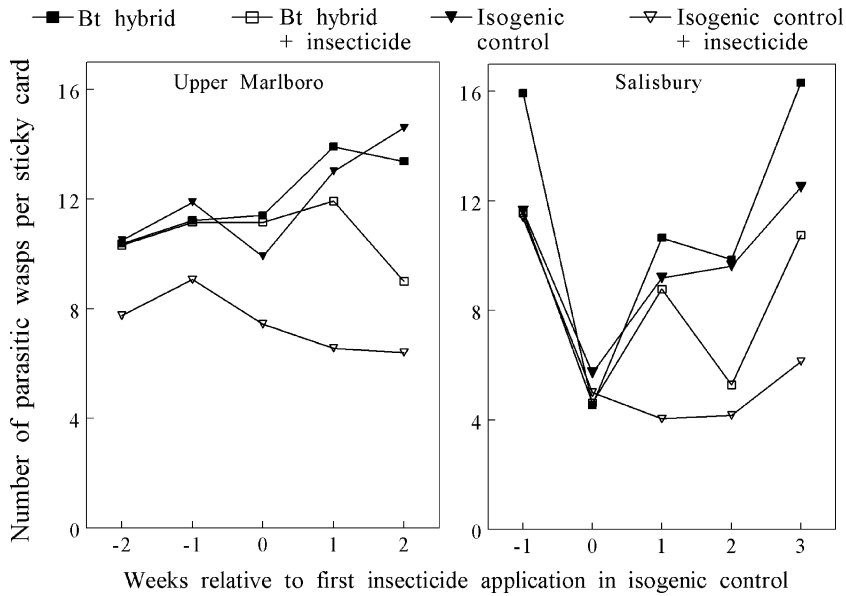


Fig. 8. Mean number of parasitic hymenopterans recorded by sticky cards in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

effect at Upper Marlboro ( $F_{(3,12)} = 4.5$ ,  $P = 0.02$ ) and a significant treatment-by-date effect at Salisbury ( $F_{(12,7.3)} = 9.31$ ,  $P = 0.003$ ). Parasitoid populations in the *Bt* plots treated once and non-*Bt* plots treated five times were reduced by 17 and 47%, respectively. At both locations, however, the numbers of parasitoids captured on sticky cards in *Bt* plots were not significantly different from captures in the control plots.

Sticky cards also provided information on adult dipterans that were active in the plant canopy. Most flies were saprovores attracted to wet deposits of pollen and anthers in leaf axils, frass, and organic matter on the surface litter. However, some families included plant feeders and parasitoids as larvae. Frit flies (Chloropidae), picture-winged flies (Otitidae), and hump-backed flies (Phoridae) were the most abundant, with mean densities ranging from 9 to 50 flies per card. PRC weights of all dipteran families were positive and thus responded in the same direction as the insecticide treatment effects. As a pooled group, overall densities were significantly higher and increased over time at Upper Marlboro compared with lower, declining populations at Salisbury (Fig. 9). Post-treatment densities of flies were significantly reduced by 31–56% in the insecticide-treated plots but were unaffected in the *Bt* plots compared with the control (Upper Marlboro treatment-by-time effect:  $F_{(12,12)} = 3.43$ ,  $P = 0.02$ ; Salisbury main effect:  $F_{(3,28.1)} = 7.07$ ,  $P = 0.001$ ). Fungus beetles (mainly Corylophidae and Cryptophagidae) also were reduced by insecticide treatments at both locations (Upper Marlboro:  $F_{(12,12)} = 5.18$ ,  $P = 0.004$ ; Salisbury  $F_{(12,12)} = 4.0$ ,  $P = 0.01$ ; treatment-by-time effects) but were not affected by *Bt* exposure.

The diversity of predatory arthropods captured by sticky cards was comparable to the predator

community active on the corn plants. Mean densities of predatory beetles, pooled over the families Coccinellidae, Staphylinidae, Lampyridae, and Cantharidae, were reduced by 27% after insecticides were applied at Upper Marlboro ( $F_{(3,8.1)} = 22.22$ ,  $P < 0.001$ ) but were not significantly affected at Salisbury. Predatory bugs, primarily insidious flower bugs (Anthocoridae) and big-eyed bugs (Lygaeidae), were numerically less abundant in the insecticide-treated plots at both locations but the differences were not significant. None of the predatory arthropods recorded on sticky cards displayed density changes over time that would indicate an adverse effect caused by *Bt* protein exposure.

Thrips, leafhoppers, aphids, flea beetles, and plant bugs were consistently the most abundant herbivores captured on sticky cards. In accordance with the high positive PRC weights, populations of plant bugs (Miridae) and leafhoppers (Cicadellidae) were significantly reduced by the insecticide treatments at Upper Marlboro ( $F_{(3,12)} = 8.25$ ,  $P = 0.003$  and  $F_{(3,11.3)} = 4.08$ ,  $P = 0.03$ ; respectively) and Salisbury ( $F_{(3,31)} = 7.57$ ,  $P < 0.001$  and  $F_{(3,33.3)} = 3.14$ ,  $P = 0.04$ ; respectively). Overall densities of flea beetles also were 38% less in the insecticide-treated plots compared with the control population, but the differences were not significant. In contrast, sticky card captures of thrips and aphids generally increased after the insecticides were applied. However, the treatment-by-time response was only statistically significant for thrips at Upper Marlboro ( $F_{(12,8.93)} = 5.86$ ,  $P = 0.006$ ). Captures of collembolans also showed increased activity associated with the insecticide applications but counts were too variable to test for significance.

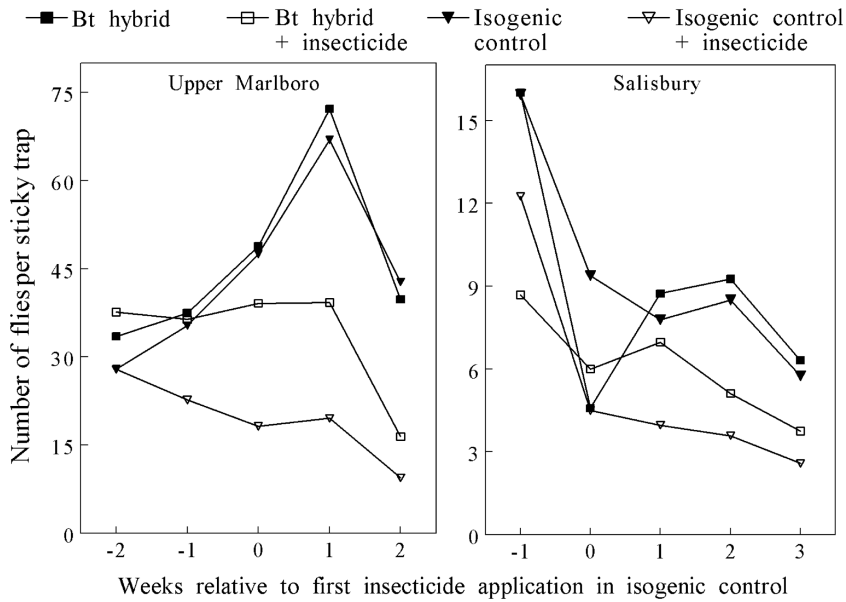


Fig. 9. Mean number of dipterans recorded by sticky cards in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

**Surface-Dwelling Community**

**Diversity.** Pitfall traps captured 73 taxa of epigeal arthropods, of which decomposers and predators comprised >90% of the total community. The diversity of taxa did not change significantly over the growing season at either location. However, the number of taxa recorded in 2001 was generally higher than the number recorded the previous year, particularly at Salisbury, where carryover residue left by the previous corn and winter cover crops increased structural diversity. There were no significant treatment or time effects on the S-W indices in either year at both locations, except for a treatment-by-time interaction effect in 2001 at Salisbury ( $F_{(9,36)} = 3.98, P = 0.001$ ). Although it was not possible to statistically test for differences in diversity between locations, the total abundance of arthropods captured in pitfall traps in no-tilled plots at Upper Marlboro was 2.2-fold higher than the total captured in conventional-tilled plots at Salisbury.

**Response Curves.** PRC curves in Fig. 10 show how the surface-dwelling communities responded over time in the *Bt* and insecticide-treated plots. At Upper Marlboro, the PRC response captured 26.6% of the variation caused by the treatment effect in the first axis and depicted overall treatment effects that were significantly different from the control community ( $F = 5.27, P = 0.009$ ). At Salisbury, the first axis of the PRC curves explained 42.0% of the treatment effect and also depicted significant departures of the treated communities ( $F = 7.66, P = 0.001$ ). Monte-Carlo permutations testing only the *Bt*-exposed communities showed no significant departures in the weighted abundances of taxa compared with the control community. Community responses in the *Bt*-treated plots were also not significant when tested

against the control, although negative departures occurred after the single insecticide application at both locations. Significant perturbations of arthropod communities in the non-*Bt* plots treated five times with insecticides were clearly visualized by PRC. At both locations, the insecticide-treated communities showed trends toward recovery after ~2 wk elapsed from the first application.

As indicated by higher positive weights, populations of staphylinids, crickets, spiders, and coccinellids were the most adversely affected by the insecticide treatments. The carabid genera *Pterostichus*, *Stenolophus*, and *Amara* also contributed significantly to changes in community responses, but these beetles increased in the treated plots when insecticides were applied, according to the negative weights. Collembolans consistently had the largest negative weights at both locations and thus also increased in numbers when insecticides were applied.

**Effects on Specific Taxa.** Pitfall traps provided the best estimations of epigeal predators, namely ants, spiders, carabid beetles, and staphylinids. As the taxon weights indicated, these predatory groups either were not affected or responded in different directions to the treatment effects. Ants did not contribute significantly to the PRCs, yet they were abundant in the surface-dwelling community, particularly in the no-tilled plots at Upper Marlboro. ANOVA results indicated no significant main or interaction effects on ants at Upper Marlboro. In contrast, ant captures in pitfall traps at Salisbury were 10-fold less because of the absence of surface residue, but were significantly higher in the non-*Bt*-treated plots ( $F_{(9,33)} = 6.79, P = 0.01$ ). Spiders, primarily lycosids, were consistently captured at both

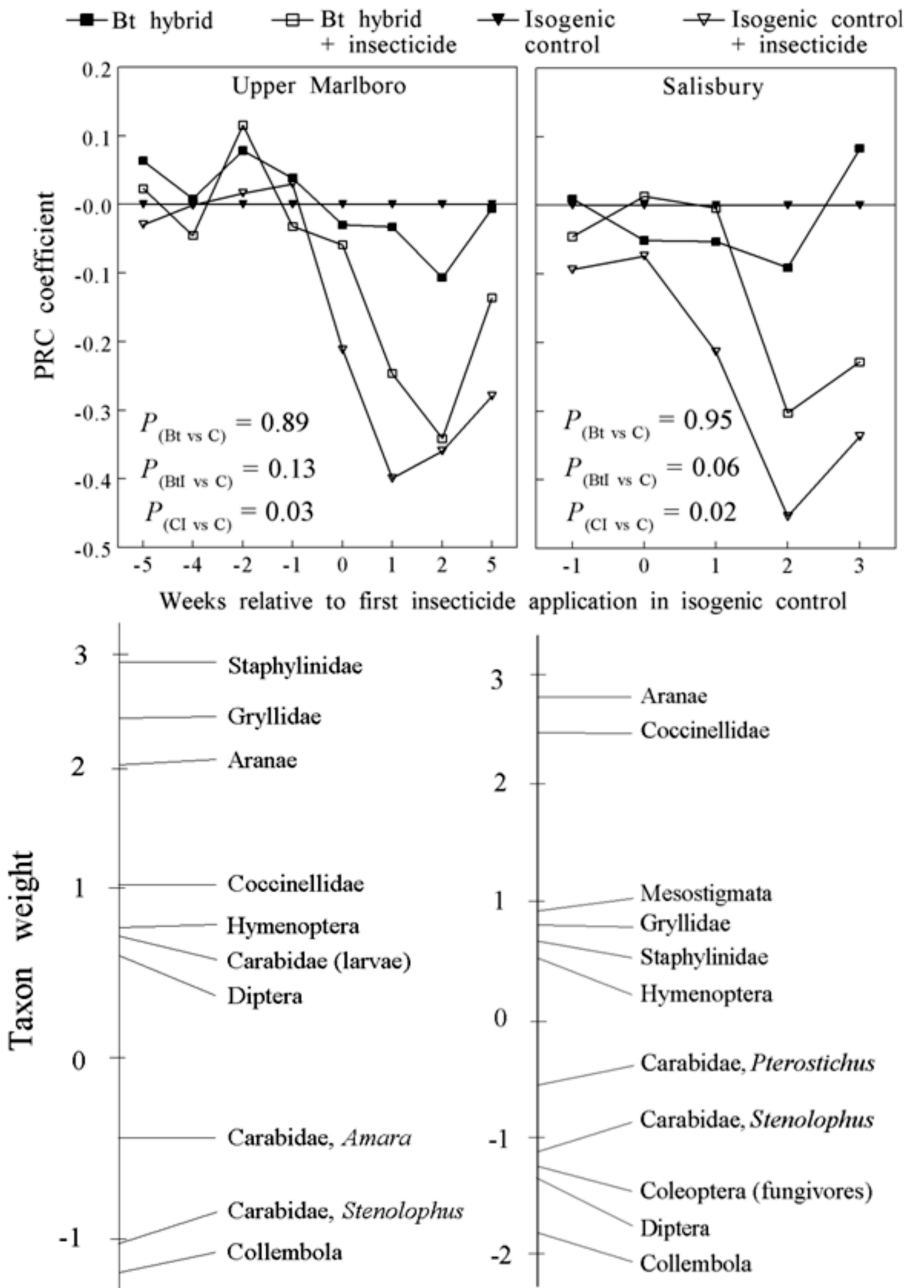


Fig. 10. PRCs and taxon weights of surface-dwelling arthropod communities exposed to *Bt* and insecticide-treated sweet corn compared with the isogenic control. Responses of taxa with positive weights followed the PRC patterns, whereas those with negative weights showed the opposite pattern. Taxa with weights between  $-0.5$  and  $0.5$  are not shown.

locations, reaching peak densities (15–25 per pitfall trap) several weeks before silking (Fig. 11). Spider activity declined naturally after silking in all plots but at a significantly greater rate in the insecticide-treated plots (Upper Marlboro:  $F_{(21,84)} = 2.26, P = 0.005$ ; Salisbury:  $F_{(12,48)} = 3.78, P < 0.001$ ; treatment-by-time effects). Staphylinid populations were 10-fold higher in the no-tilled plots at Upper Marlboro compared with overall populations at Salisbury (Fig. 12). At both

locations, mean densities were reduced by 39 and 67% in the *Bt* and non-*Bt* insecticide-treated plots, respectively, but this interaction effect was only significant at Upper Marlboro ( $F_{(21,84)} = 2.26, P = 0.005$ ). Certain carabid genera exhibited positive responses to the insecticide treatments; however, data were too variable to test for significance. The seed-feeding carabids in the genus *Amara* were captured primarily during the first sampling date at Upper Marlboro (averaging

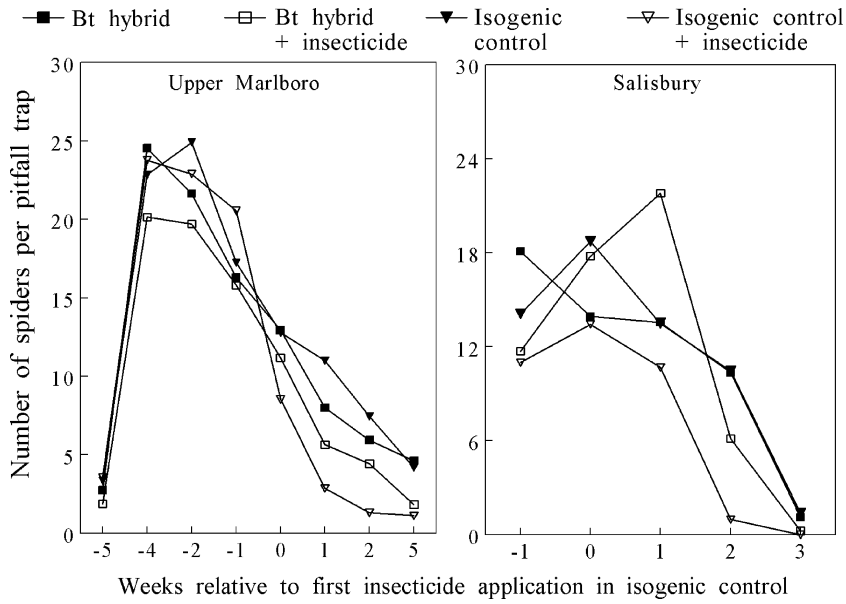


Fig. 11. Mean number of spiders recorded by pitfall traps in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

5.6 per trap in 2000), but were rare at Salisbury. Pooled densities of all carabids that function chiefly as predators were not significantly affected by the treatments at either location. None of the predatory taxa described above were significantly affected by exposure to *Bt* corn, nor was any population trend evident that might suggest an adverse effect.

Collembolans and oribatid mites were by far the predominant decomposing organisms recorded in pitfall samples, followed by field crickets and a variety of dipterans and fungivorous beetles. Mite abundance was 64-fold higher in the no-tilled plots at Upper Marlboro compared with densities at Salisbury. Despite their high abundance, mites did not contribute to

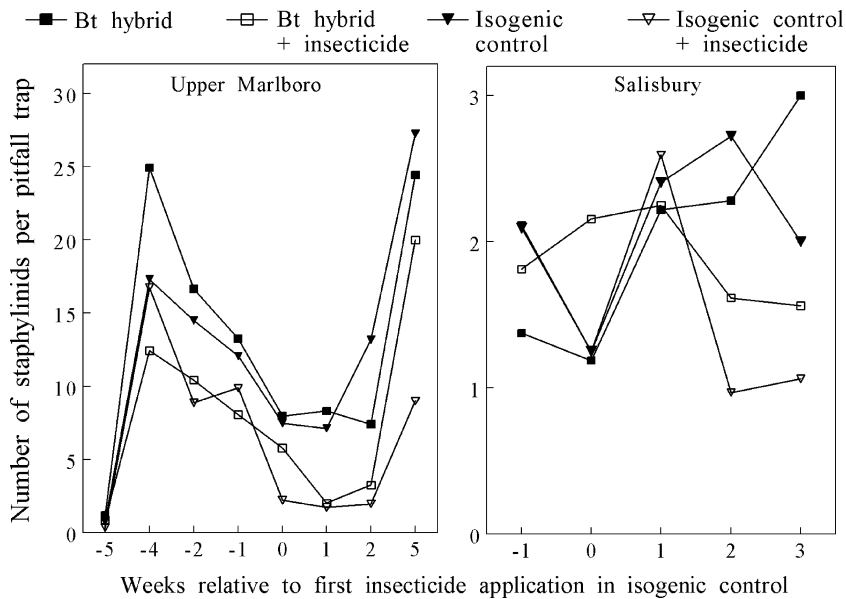


Fig. 12. Mean number of staphylinids recorded by pitfall traps in *Bt*, insecticide-treated, and isogenic control plots at Upper Marlboro and Salisbury, MD. Data pooled and averaged over years relative to weekly intervals before and after the first insecticide application in isogenic control (week 0).

the overall community responses described by the PRCs and were not significantly affected by treatments. Similar to mites, densities of collembolans were about five-fold higher at Upper Marlboro in response to food sources provided by the surface residue in the no-tilled plots. Collembolans accounted for 53% of the total pitfall captures and contributed significantly to changes in the overall community responses as indicated by the larger negative PRC weights. Springtails were not affected by the *Bt* hybrid. However, the combined densities of all families of Collembola responded positively to the insecticide treatments (Upper Marlboro:  $F_{(3,9)} = 8.14$ ,  $P = 0.006$ ; Salisbury:  $F_{(3,9)} = 4.52$ ,  $P = 0.03$ ). Overall activity in pitfall traps after insecticides were applied increased by 8% in the *Bt*- and non-*Bt*-treated plots.

### Discussion

This study is the first community-level assessment of the potential nontarget effects of Cry1Ab expression in *Bt* sweet corn compared with conventional insecticide control. Of the total abundances of 128 taxonomic groups recorded, 39, 32, 24, and 5% were comprised of decomposers, predators, herbivores, and parasitoids, respectively. There were no significant differences in diversity and community-level responses, as depicted by the PRCs, associated with the presence and absence of Event Bt11 sweet corn. Results from all sampling methods clearly established that potential exposure to Cry1Ab protein had no adverse impact on major taxa of predators and parasitoids. Our results agree with other field studies that found no significant nontarget effects from the lepidopteran-active Cry proteins (Pilcher et al. 1997, Lozzia 1999, Candolfi et al. 2003, Jasinski et al. 2003, French et al. 2004, Sisterson et al. 2004, Dively 2005, Naranjo 2005, Whitehouse et al. 2005, Torres and Ruberson 2005, Head et al. 2005, Daly and Buntin 2005, Pilcher et al. 2005). Direct adverse effects on parasitoids were not expected given the known spectrum of activity of the Cry1Ab protein. However, Pilcher et al. (2005) reported that a specialist braconid of European corn borer was negatively affected in *Bt* corn because the host population was significantly reduced. Low densities of specialist parasitoids of target insects in this study precluded a statistical determination of this effect. The abundance of major decomposers, collembolans and oribatid mites, was also unaffected by *Bt* sweet corn. These results are consistent with laboratory and field studies that found no adverse effects of exposure to either Cry1 or Cry3 proteins on springtails and other decomposers (Yu et al. 1997, Sims and Martin 1997, Al-Deeb et al. 2003, Bhatti et al. 2005, Bitzer et al. 2005).

Because it is labor-intensive to examine all potential nontarget organisms associated with transgenic sweet corn, this study provides a database for selecting nontarget beneficial taxa that could serve as bioindicators for future work. The major foliage-dwelling predators were anthocorids [mainly *Orius insidiosus* (Say)], chrysopids [*Chrysoperla carnea* (Stephens)], and coccinellids [predominately *Coleomegilla maculata* (DeGeer)], all known to play a major role in natural control of

many insect pests in corn agroecosystems (Coll and Bottrell 1992). Populations of these insects were sufficiently abundant to allow statistical analysis, particularly when their numbers peaked during peak pollen shed. These predators also are good candidates for monitoring nontarget effects because they are potentially exposed to *Bt* proteins through multiple routes of exposure—direct contact with *Bt* toxins by feeding on pollen or plant sap (only in the case of *O. insidiosus*) and secondary exposure by feeding on herbivorous insects that have fed on the *Bt* plant. Plant inspections provided the most consistent data on the life stages of these predator groups, whereas the sticky cards captured primarily adult predators and were vulnerable to exaggerated captures of excited arthropods after insecticide applications. Also, changes in predator populations over time determined by plant inspections were more representative of the actual population densities.

Pitfall trapping has become the standard method in nontarget studies for sampling epigeal predators in the soil-litter community. In this study, prominent surface-dwelling predators were ants, spiders (mainly lysocids), staphylinids, and carabids. Of these, selection of the most appropriate bioindicators depends on whether the nontarget study system is conventionally or no-tilled. Two species of ants (*Aphaenogaster rudis* Emery and *Myrmica americana* Weber) were commonly found in relatively high numbers at Upper Marlboro but were scarce in the conventionally tilled plots at Salisbury. Moreover, ants may not be reliable indicators because of their nonrandom spatial distribution in cornfields, which can lower the statistical power for detecting differences. Ants also exhibit variable feeding habits, so their ecological role in food web interactions may be difficult to interpret without identification to the species level. Carabids and staphylinids have been recommended as bioindicators to assess the ecological risks of the rootworm-resistant *Bt* corn (EPA 2002). The composition of carabid beetles at the genus level varied by sampling date and tillage system but consistently included *Harpalus*, *Stenolophus*, *Chlaenius*, and *Pterostichus*. Carabid densities in the no-tilled plots were two-fold greater than the densities in conventionally tilled plots. The species *Pterostichus lucublandus* Say, *Chlaenius nemoralis* Say, and *Harpalus pennsylvanicus* DeGeer were common predatory species that occurred during silking; thus, these species would be appropriate indicators of potential treatment effects. Like ants, staphylinid populations were favored by the presence of surface litter. Pitfall captures of rove beetles were 10 times higher in the no-tilled plots and 4 times more numerous than carabids at Upper Marlboro. In the conventionally tilled plots at Salisbury, rove beetles were relatively low and ranked second to carabids. The taxonomy of Staphylinidae is difficult; thus, predominant species could not be determined. However, the majority of rove beetles belonged to the genera *Aleochara*, *Lep-tusa*, and *Myllaena*.

The families Mymaridae, Trichogrammatidae, and Scelionidae were the most frequently captured para-

sitoids on sticky cards. Most species parasitize eggs of insects and other arthropods, thus they would not be appropriate indicators for measuring effects of the *Bt* toxins, because they are unexposed and not affected by density changes in target pest populations. Tachinid flies and braconid wasps have been studied as specific nontargets in *Bt* crops (Orr and Landis 1997, Venditti and Steffey 2002, Pilcher et al. 2005). However, these insects were not abundant enough in this study to statistically test for treatment effects.

For beneficial surface-dwelling decomposers, springtails have been a standard indicator group for testing nontarget effects of pesticides and *Bt* crops (Jepson et al. 1994, EPA 2000, 2002). Pitfall captures of collembolans and mites were 5–64 times greater at Upper Marlboro where the decomposing litter provided fungal growth and moist micro-environmental conditions for population growth. In general, both above-ground and surface-dwelling arthropods increase in abundance as tillage is decreased in a cropping system (Stinner and House 1990). Thus, nontarget field studies on decomposers and other trophic levels would be better conducted in a no-tillage system to maximize the populations exposed and reduce sampling variance.

The pyrethroid insecticide applied to the *Bt* and non-*Bt* corn plots caused significant reductions in the abundance and diversity of many arthropods. In all cases, PRC responses of the pyrethroid-treated communities showed significant departures from the non-*Bt* control, which coincided with insecticide applications. One insecticide application in the *Bt* plots reduced communities of natural enemies by 21–48%. Five applications in the non-*Bt* plots reduced natural enemy communities by 33–70%. All plant-dwelling predators, parasitoids, decomposers, and most herbivores were sensitive in varying degrees to the pyrethroid insecticide. The surface-dwelling arthropods were generally less affected because of the protective shelter afforded by the surface litter and lower amounts of insecticide residue that settled on the litter surface. Other field studies have reported significant reductions in the diversity and abundance of nontarget communities in conventionally treated fields compared with more diverse communities in *Bt* cotton and potato fields (Head et al. 2001a, b, Reed et al. 2001, Naranjo and Ellsworth 2002, Fitt and Wilson 2003, Bhatti et al. 2005, Dively 2005). A few taxa also were affected positively by the insecticide treatments. Collembola, mites, and aphids increased in numbers in the insecticide-treated plots. Resurgence of these arthropods often occurs after foliar and soil applications of pyrethroid insecticides (Bitzer et al. 2005) as a result of disruption of natural enemy populations. In this study, a consistent reduction in staphylinid abundance in insecticide treated plots was the most likely cause for the springtail response. Nontarget communities in *Bt* plots treated once with an insecticide exhibited some recovery depending on the particular nontarget group, but communities exposed to five applications showed no trends toward recovery during the crop cycle. Whether these ecological disturbances carryover to the following season or have landscape-scale consequences remains unknown.

In summary, this study clearly showed that the nontarget effects of *Bt* transgenic sweet corn on natural enemies and other arthropods were significantly far less than the community-level disruptions of conventional insecticide control. The weight of evidence supports the general consensus that there are no unexpected ecological risks caused by transgenic lepidopteran-resistant corn on nontarget organisms. The use of *Bt* sweet corn can result in significant reductions in conventional insecticides and thus fewer disruptions to beneficial insects, which may lead to enhanced natural control of secondary pests (Betz et al. 2000).

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