

Conventional and Seed-Based Insect Management Strategies Similarly Influence Nontarget Coleopteran Communities in Maize

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ABSTRACT Seed-based pest management tools, such as transgenes and seed treatments, are emerging as viable alternatives to conventional insecticide applications in numerous crops, and often occur as coupled technologies. Seed-based technologies have been readily adopted in maize, for which ecological studies are needed to examine effects to farmland biodiversity. We compared the response of nontarget coleopteran communities in Cry1Ab/c sweet corn and Cry3Bb field corn to conventional pyrethroid applications and a control. Of particular interest was the Cry3Bb field corn, which was coupled with a neonicotinoid seed treatment and was not rotated across years. A functionally diverse subset of the coleopteran community, consisting of three families (Carabidae, Chrysomelidae, and Nitidulidae) and 9,525 specimens, was identified to species. We compared coleopteran diversity and dynamics using rarefaction and ordination techniques. There were no differences in species richness among treatments; however, higher activity densities were more common in the control. In the nonrotated field corn, principal response curves showed a consistent pattern of treatment communities deviating from the control communities over time, whereas crop rotation in the sweet corn negated treatment effects. Treatment effects could not be detected when beetles were grouped based on functional roles. Results indicate that neonicotinoid seed-based treatments may have effects on some nontarget coleopterans, but these effects are similar to conventional pyrethroid applications.

KEY WORDS Bt maize, Coleoptera, neonicotinoid seed treatments, nontarget effects

Many insect pest management decisions in agriculture are now made during the purchase of seed. Options include an array of transgenic technologies and seed treatments that can serve to augment, or even replace, conventional insecticide applications. The ecological ramifications of these seed-based delivery mechanisms, including effects to nontarget organisms and biodiversity, are the topic of ongoing testing and debate. Much attention has been directed toward genetically engineered crops containing *Bacillus thuringiensis* (*Bt*) transgenes that encode for insecticidal Cry proteins (Naranjo et al. 2005). Although this pest management approach is considered more localized and/or target-specific than conventional foliar or soil-applied insecticides, numerous studies have been conducted to examine potential threats to nontarget insects. To date, most studies have shown that the use of *Bt* crops can result in more efficient control of pest taxa and less reliance on conventional insecticides (Musser and Shelton 2003, Brookes and Barfoot 2006),

and may promote or sustain on-farm biodiversity because of reductions in insecticide use (Cattaneo et al. 2006, Leslie et al. 2007).

Transgenic technology has been widely adopted in maize and cotton. In maize, *Bt* transgenes expressing Cry1- and Cry3-based proteins have been used for control of European corn borer [*Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae)] and corn rootworm [*Diabrotica* spp. (Coleoptera: Chrysomelidae)], respectively. Ecotoxicological studies have concluded that, under field conditions, adverse effects to nontarget organisms are nonexistent, minimal, or cannot be detected for both Cry1 (Orr and Landis 1997, Pilcher et al. 1997, Wraight et al. 2000, Floate et al. 2007, Hoheisel and Fleischer 2007) and Cry3 (Al-Deeb and Wilde 2003, Ahmad et al. 2005, Bhatti et al. 2005) maize, except in instances in which the abundance of a specialist parasitoid of the target pest taxa is reduced because of lack of prey (Pilcher et al. 2005). Broader conclusions have been drawn from nontarget studies in *Bt* crops by using meta-analyses (Marvier et al. 2007, Naranjo 2009). In general, nontarget effects have been negligible, even when examining specific groups, such as biological control agents (Romeis et al. 2006) and other functional guilds (Wolfenbarger et al. 2008; but also see Lundgren et al. 2009 for a review of the interactions among ecological pathways and biological control in transgenic crops).

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Realistically, however, transgenic crops often come coupled with other seed-based technologies, including insecticidal seed treatments. For example, all commercially available Cry3-based transgenic corn seed is treated with systemic neonicotinoids (Smith et al. 2004). Although much effort has been made to isolate and examine the effects of transgenes in *Bt* maize, neonicotinoid seed treatments may pose a greater threat to nontarget organisms than *Bt* toxins. Coleopterans comprise a wide range of feeding guilds (seed predators, omnivores, detritivores, predators, herbivores and even parasitoids) that may allow them to come in direct or indirect contact with these toxins in a field setting. Seeding rates for maize vary with the yield potential of soil. In Pennsylvania, maize grown for grain range from 26,000–30,000 seeds per acre, and reach 36,000 for silage corn; rates may be higher in soils with greater yield potential. Thus, the potential for nontarget exposure could arise from the product leaching into the soil, direct feeding on the plant where the systemic insecticide is being expressed, or indirectly by feeding on corn-feeding herbivores. In laboratory bioassays, Mullin et al. (2005) fed nontarget carabids on transgenic (Cry3Bb and Cry1Ab/c) corn pollen and seedlings germinated from seeds treated with systemic neonicotinoids. This study concluded that neonicotinoid seed treatments, and not Cry3Bb, represent a major mortality factor for carabid beetles. Additionally, guttation drops collected from corn seedlings germinated from neonicotinoid-coated seeds were recently shown to be toxic to honey bees in laboratory feeding trials (Girolami et al. 2009). Field experimentation must consider the effects of these broader systems for realistic evaluation of currently deployed transgenic crops.

Among the variety of approaches for examining community-level nontarget effects of pest management practices, there remains a need for more studies with species-level resolution (Danks 1996). However, species-level identification often requires large investments of time and money, which can be exacerbated by the current bottleneck in taxonomic expertise (Kim and Byrne 2006, Rohr et al. 2007). Because sampling insect communities results in large numbers of specimens that often cannot all be identified to species, community analyses are often done at higher taxonomic levels, such as families (Rohr et al. 2007). However, species-level identification provides the most accurate ecological inference (Schmidt-Kloiber and Nijboer 2004), as insect families often contain numerous species representing a wide range of feeding habits (Danks 1996). Species-level identification also allows for diversity measurements, such as species richness and dominance structure, and for the determination of which species, if any, are driving community shifts among treatments (Lundgren et al. 2009). To perform a community-based assessment with species-level resolution, our study focuses on a functionally diverse subset of the nontarget community in question, for which species-level taxonomic resolution can be achieved, and which can reasonably be pre-

sumed to be at risk from direct or indirect effects of pest management strategies in maize.

Using a systems-based approach, we examined the response of nontarget coleopterans to current insect pest management practices in sweet corn and field corn in Pennsylvania, including *Bt* field corn containing neonicotinoid seed treatments. For each type of maize, we used a 2×2 factorial experiment comparing systems with or without seed-based insect control tactics, and with or without conventional pyrethroid applications during the growing season. Carabidae activity density and species richness were compared among treatments. To examine the effect to the broader coleopteran community, we analyzed the dynamics of species from three families (Carabidae, Chrysomelidae, and Nitidulidae) representing diverse functional roles. Seed-based technology in field corn was of primary concern because of the presence of neonicotinoid seed treatments shown to be highly toxic to carabid beetles in laboratory bioassays (Mullin et al. 2005).

Materials and Methods

Experimental Design. We used a randomized complete block (RCB) factorial experiment to examine the effects of seed-based technologies and insecticide inputs on coleopteran communities in sweet corn and field corn. Earlier work (Leslie et al. 2009) describes the population-level response of four dominant species within the Carabidae. Here we focus on overall carabid diversity and community-level response of species from three functionally diverse families. Thus, some details of field plots, treatments and sample collections are more extensively covered in Leslie et al. (2009). The experiment was conducted at the Russell E. Larson Agricultural Experimental Station in Rock Springs, PA, and spanned the 2003 and 2004 growing seasons. The four treatments were a 2×2 factorial defined by presence/absence of seed technologies and presence/absence of conventional pyrethroid insecticides. In each year, we used 12 experimental plots (each 57.7×19.2 m) of sweet corn and field corn, representing the four treatments replicated three times. Snap beans were planted adjacent to experimental plots to provide a rotational crop for sweet corn in the second year. Field corn was not rotated, as is common practice for those growing transgenic cultivars in the northeastern United States.

In sweet corn, the seed technology consisted of a transgene expressing Cry1Ab/c toxins (*Tra*) versus its isoline (*Iso*), and the conventional insecticide management consisted of four pyrethroid applications during the silking stage (*PyrSilk*). The four treatments are thus labeled as: (1) no insect control inputs (*Iso*), (2) seed technology only (*Tra*), (3) insecticides only (*PyrSilk*), and (4) a combination of seed technology and insecticide applications (*Tra/PyrSilk*). For field corn, the seed technology consisted of a neonicotinoid seed treatment and a transgene expressing Cry3Bb1 toxins (*TraNeo*) versus its isoline (*Iso*), and the conventional insecticide management consisted of a soil-

directed, at-planting application of a pyrethroid (*PyrSoil*). The four field corn treatments are described as: (1) no insect control inputs (*Iso*), (2) seed technology only (*TraNeo*), (3) conventional insecticides only (*PyrSoil*), and (4) a combination of seed technology and conventional insecticides applications (*TraNeo/PyrSoil*).

In the conventional insecticide management treatments, sweet corn received four late-season foliar applications of the pyrethroid lambda-cyhalothrin (Warrior 1 EC, Syngenta, Greensboro, NC, 11.4% [AI], 222 ml/ha) to control ear-feeding lepidopterans. Conventionally managed field corn received an at-planting soil application of the pyrethroid tefluthrin (Force 3G, Syngenta, Greensboro, NC, 3% [AI], 3.7 kg/ha) to control corn rootworm and other seed- and root-feeding insects.

In the seed-based management treatments, sweet corn expressed Cry1Ab/c toxins. The field corn expressed Cry3Bb1 endotoxins and the seed was coated with a neonicotinoid seed treatment (160 μ g imidacloprid/seed, or 250 μ g clothianidin/seed, Bayer CropScience, Research Triangle Park, NC). The near isolines of sweet corn and field corn did not contain transgenes or insecticidal seed treatments.

Insect Collection and Identification. Pitfall traps (14.1 cm deep with 10.9 cm internal diameter [i.d.], inserted flush with the ground) were used to capture beetles. Another small collection cup (5.5 cm deep, 8.2 cm i.d.) filled with ethylene glycol was placed inside the trap for removal of specimens. Traps were arranged in transects of five, placed at 9, 18, 27, 36, and 45 m within the rows of all field corn and sweet corn plots. Trapping occurred every 7–14 d and consisted of 10 sampling dates (12 June–15 Sept) in 2003 and 11 dates (5 June–27 Sept) in 2004. For each sampling date, traps were opened for 72 h in each field and sweet corn plot for a total of 120 open traps. Beetles were transferred to ethanol, sorted, pinned, and uniquely labeled. Species level identifications were made by Robert Davidson (Carnegie Museum of Natural History, Pittsburgh, PA), David Biddinger (Pennsylvania State University), and Timothy Leslie (Pennsylvania State University), using several keys (Wilcox 1954, Dillon and Dillon 1961, Downie and Arnett 1996, Ciegler 2000) and voucher specimens from previous studies (Hoheisel 2002, Leslie et al. 2007). Beetles were assigned to functional groups based on feeding ecology records for each species (see Dillon and Dillon 1961, Capinera 2001, Larochelle and Larivière 2003, Lundgren 2009). Voucher specimens are housed in the Department of Entomology, Pennsylvania State University.

Analyses. Data were recorded as activity-density (no. beetles/pitfall trap/72 h) for each species at the plot-level. The pitfall traps were primarily directed toward epigeal coleopterans, of which Carabidae were captured in the highest numbers. Thus, carabid species richness and activity density was compared among the four treatments. We compared interpolated species richness using rarefaction curves (Gotelli and Colwell 2001) generated with EstimateS

v. 7.5 (Colwell 2005). Rarefaction curves depict the statistical expectation of species accumulation as sampling effort increases and are generated by iteratively resampling the sample-by-species abundance matrix (in which a Monte Carlo permutation procedure randomly reorders the samples in the matrix). The resulting smoothed species accumulation curve depicts the cumulative number of species expected per sampling effort. Comparing species richness between treatments can then be done at a standardized sampling effort (Gotelli and Colwell 2001). Analyses were performed for each crop within each year. Rarefaction curves were individual-based and were generated from 99 permutations of the samples. Analysis of variance (ANOVA) in JMP v.7 (SAS Institute Inc., 2007) was used to examine the effect of treatments on carabid activity densities within each crop and year and included the main effects of seed technology (presence/absence) and conventional pyrethroid insecticides and their interactions. Significance was set at $P = 0.05$. Tukey mean comparisons were used to determine significance among the four treatment levels defined by the interaction.

To determine treatment effects on the broader coleopteran community, we examined species from three families that were abundant in trap captures and encompassed numerous functional roles in maize agroecosystems: (1) Carabidae – predators/omnivores/weed seed predators, (2) Chrysomelidae – herbivores/corn feeders, and (3) Nitidulidae – corn feeders/detritivores. We measured changes in community composition over time, and associations between treatments and functional groups, using several ordination techniques in CANOCO 4.5 (Ter Braak and Šmilauer 2002).

Principal response curve (PRC) analyses (Van den Brink and Ter Braak 1998) were developed using CANOCO 4.5 to determine how the coleopteran community responded to treatment variables over time relative to control plots. PRC is a constrained ordination approach where the explanatory variables are the interaction of the treatment variables with each sampling point in time (Leps and Šmilauer 2003). In our analysis, we identified the isolate maize receiving no insecticides (*Iso*) as the control treatment, and its coleopteran community was represented as a horizontal line over time. The canonical coefficients for the communities in the remaining treatments were plotted over time and graphically represented their deviation from this control community. The significance of this deviation was tested using Monte Carlo permutations.

PRCs were performed using the species with highest activity densities (those representing >2% of total catch of each family in each year) from each of the three families and were done separately for sweet corn and field corn because the two crops differed in pest management inputs and rotation. Data were Hellinger-transformed (Legendre and Gallagher 2001) before analysis. The significant effect of block (field corn: $F(34, 154) = 1.60$; $P = 0.029$; sweet corn: $F(34, 154) = 1.62$; $P = 0.026$) was controlled for before the

Table 1. Species list for three coleopteran families collected at Pennsylvania State University research farms in Rock Springs, PA, during 2003 and 2004

Carabidae	% Total	Function	Carabidae cont.	% Total	Function
<i>Harpalus pensylvanicus</i> DeGeer	19.02	S, O	<i>Cyclotrachelus furtivus</i> (LeConte)	0.01	P
<i>Scarites quadriceps</i> Chaudoir	12.27	P	<i>Microlestes pusio</i> LeConte	0.01	P
<i>Poecilus chalcites</i> (Say)	11.05	P, S			
<i>Bembidion quad. opp.</i> Say	8.13	P	Chrysomelidae		
<i>Pterostichus melanarius</i> Illiger	7.97	P, S	<i>Chaetocnema pulicaria</i> Melsh.	38.16	C
<i>Chlaenius t. tricolor</i> Dejean	4.96	P	<i>Chaetocnema cribrifrons</i> LeConte	30.54	H
<i>Bembidion rapidum</i> (LeConte)	4.24	P	<i>Chaetocnema minutis</i> Melsh.	11.92	H
<i>Dyschirius globulosus</i> (Say)	3.50	P	<i>Psylliodes punctulata</i> Melsh.	4.22	H
<i>Poecilus lucublandus</i> (Say)	2.87	P, S	<i>Disonycha xanthomelas</i> (Dalman)	2.07	H
<i>Harpalus herbivagus</i> Say	2.70	S, O	<i>Disonycha collata</i> Fabricius	1.99	H
<i>Agonum muelleri</i> (Herbst)	2.34	S, P	<i>Epitrix cucumeris</i> (Harris)	1.82	H
<i>Cicindela punctulata</i> Olivier	2.09	P	<i>Epitrix humeralis</i> Dury	1.74	H
<i>Anisodactylus sanctaerucius</i> (F.)	1.92	S, O	<i>Psylliodes convexior</i> LeConte	0.99	H
<i>Amara exarata</i> Dejean	1.90	S, O	<i>Chaetocnema denticulata</i> (Illiger)	0.75	C
<i>Elaphropus anceps</i> (LeConte)	1.86	P	<i>Phyllotreta striolata</i> (Fabricius)	0.58	H
<i>Harpalus affinis</i> (Schränk)	1.54	S, O	<i>Distignoptera apicalis</i> Blake	0.50	H
<i>Pterostichus stygicus</i> Say	1.24	P	<i>Epitrix hirtipennis</i> (Melsh.)	0.50	H
<i>Harpalus compar</i> LeConte	1.23	S, O	<i>Hornaltica atriventris</i> (Melsh.)	0.50	H
<i>Stenolophus comma</i> (Fabricius)	1.17	C, O	<i>Systema hudsonias</i> (Forster)	0.50	C
<i>Patrobus longicornis</i> (Say)	1.09	P, O	<i>Systema frontalis</i> (Fabricius)	0.41	C
<i>Agonum placidum</i> (Say)	1.02	P, C	<i>Disonycha triangularis</i> (Say)	0.33	H
<i>Agonum cupripenne</i> (Say)	0.68	P, C	<i>Epitrix fuscula</i> Crotch	0.33	H
<i>Clivina bipustulata</i> Fabricius	0.63	P	<i>Longitarsus melanurus</i> (Melsh.)	0.25	H
<i>Amara impuncticollis</i> (Say)	0.58	S, O, C	<i>Mantura chrysanthemii</i> (Koch)	0.25	H
<i>Harpalus rubripes</i> Duftschmid	0.58	S, O	<i>Ophraella conferta</i> (LeConte)	0.25	H
<i>Amara familiaris</i> (Duftschmid)	0.48	S, O	<i>Systema elongata</i> (Fabricius)	0.25	H
<i>Clivina impressifrons</i> LeConte	0.47	P, C	<i>Chaetocnema confinis</i> Crotch	0.17	C
<i>Amara aenea</i> (DeGeer)	0.37	S, O	<i>Chalepus dorsalis</i> Thunb.	0.17	H
<i>Colliuris pensylvanica</i> Linnaeus	0.33	P	<i>Anisostena nigrita</i> (Olivier)	0.08	H
<i>Harpalus caliginosus</i> Fabricius	0.29	S, O	<i>Colaspis brunnea</i> Fabricius	0.08	H
<i>Trechus quadristriatus</i> (Schränk)	0.29	S, P	<i>Diabrotica undecimpunctata</i> (L.)	0.08	C
<i>Harpalus erythropus</i> Dejean	0.18	S, P	<i>Diabrotica virgifera</i> LeConte	0.08	C
<i>Harpalus longicollis</i> LeConte	0.11	S, P	<i>Lema trilineata</i> (Olivier)	0.08	H
<i>Microlestes linearis</i> LeConte	0.11	P, C	<i>Longitarsus subrufus</i> LeConte	0.08	H
<i>Bembidion affine</i> Say	0.10	P	<i>Longitarsus succineus</i> (Foudras)	0.08	H
<i>Harpalus faunus</i> Say	0.10	S, O	<i>Longitarsus waterhousi</i> Kutsch.	0.08	H
<i>Bradycellus rupestris</i> (Say)	0.08	P, S	<i>Odontota dorsalis</i> (Thunberg)	0.08	H
<i>Anisodactylus harrisi</i> LeConte	0.08	S, O	<i>Phyllotreta zimmermanni</i> Crotch	0.08	H
<i>Anisodactylus rusticus</i> Say	0.07	S, O			
<i>Stenolophus ochropezus</i> (Say)	0.07	C, O	Nitidulidae		
<i>Pterostichus mutus</i> (Say)	0.07	P	<i>Stelidota geminata</i> (Say)	34.59	C, D
<i>Diplocheila obtusa</i> LeConte	0.06	P, C	<i>Glischrochilus quadrisignatus</i> (Say)	34.21	C, D
<i>Bembidion minus</i> Hayward	0.04	P	<i>Carpophilus lugubris</i> Murray	19.13	C, D
<i>Cicindela sexguttata</i> Fabricius	0.04	P	<i>Glischrochilus fasciatus</i> (Olivier)	9.43	C, D
<i>Agonum punctiforme</i> (Say)	0.03	S, P	<i>Carpophilus brachypterus</i> (Say)	2.07	C, D
<i>Calathus gregarius</i> (Say)	0.01	P, S	<i>Carpophilus dimidiatus</i> (Fabricius)	0.38	C, D
<i>Cicindela tranquebarica</i> Herbst	0.01	P			

Percent of total capture within each family and functional roles are noted next to each species. The first functional group for each species was the designation used in the ordination of functional groups (Fig. 5). P = predator, O = omnivore, S = seed predator, H = herbivore, C = corn feeder, D = detritivore.

PRC analysis, and thus the PRC was conducted on the residuals.

The PRC provides individual taxon weights to investigate how closely individual species within the community follow the overall community patterns in each treatment. A positive taxon weight indicates that the species follows the principal response curve while a negative taxon weight indicates that the species responds in the opposite fashion. The absolute value of the taxon weight indicates the strength of the relationship to the principal response, with greater absolute values indicating a stronger relationship. Species with low taxon weights (between -0.5 and 0.5) were removed from the PRC diagrams to reduce clutter and thus facilitate interpretation.

A redundancy analysis (RDA), another constrained ordination technique, was used to investigate associations between treatments and functional groups. In RDA, orthogonal axes explaining the greatest amount of variation in the response data are constrained by the treatment variables. We performed an RDA for sweet corn and field corn individually, with both years of data combined. Before analysis, each species was assigned to a functional group (see Table 1 for functional group designations), and data were summed across species within each group. In CANOCO, these data were centered and standardized, and species scores were divided by the standard deviation after axes extraction. Treatment variables were assessed using Monte

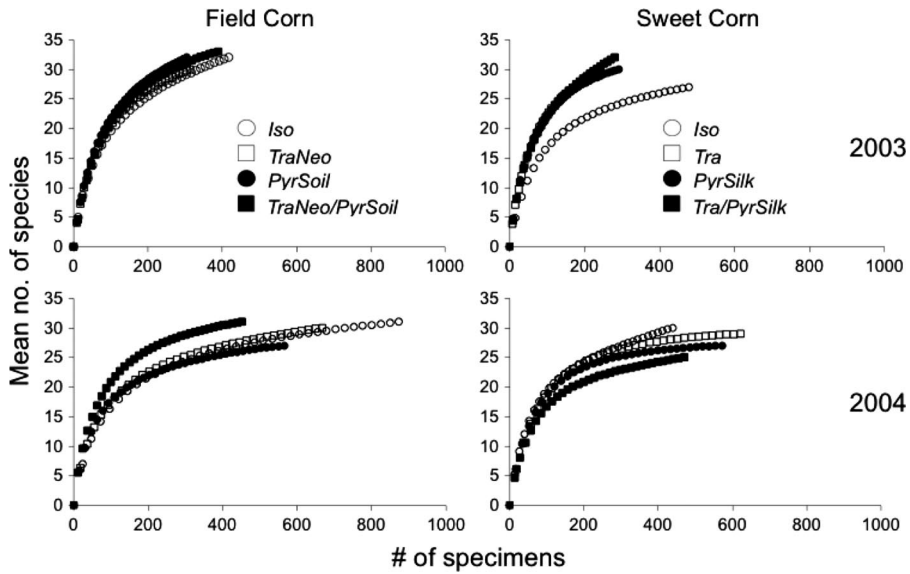


Fig. 1. Individual-based rarefaction curves showing Carabidae species accumulation among four treatments in field corn and sweet corn in 2003 and 2004.

Carlo permutations with 499 iterations and forward stepwise selection. Bi-plots diagrams were generated in CanoDraw (Ter Braak and Šmilauer 2002) and used to interpret results.

Results

Coleopteran Diversity. Twenty-six families of Coleoptera were collected from 2,760 pitfall traps during the 2-yr study. This included 7,256 Carabidae representing 49 species, 1,208 Chrysomelidae representing 34 species, and 1,061 Nitidulidae representing six species (Table 1).

Individual-based rarefaction curves were generated to compare Carabidae species richness between treatments. All individual-based rarefaction curves approached asymptotes of 25–35 species after 200–400 individuals had been collected (Fig. 1). There were no significant differences (confidence intervals overlapped) in Carabidae species richness among the four treatments in sweet corn or field corn in either year. In contrast to species richness, carabid activity density varied significantly among treatments in all crops and years, except in the first year of field corn (Fig. 2). The Iso treatments generated the highest carabid activity densities in all instances, except second year sweet

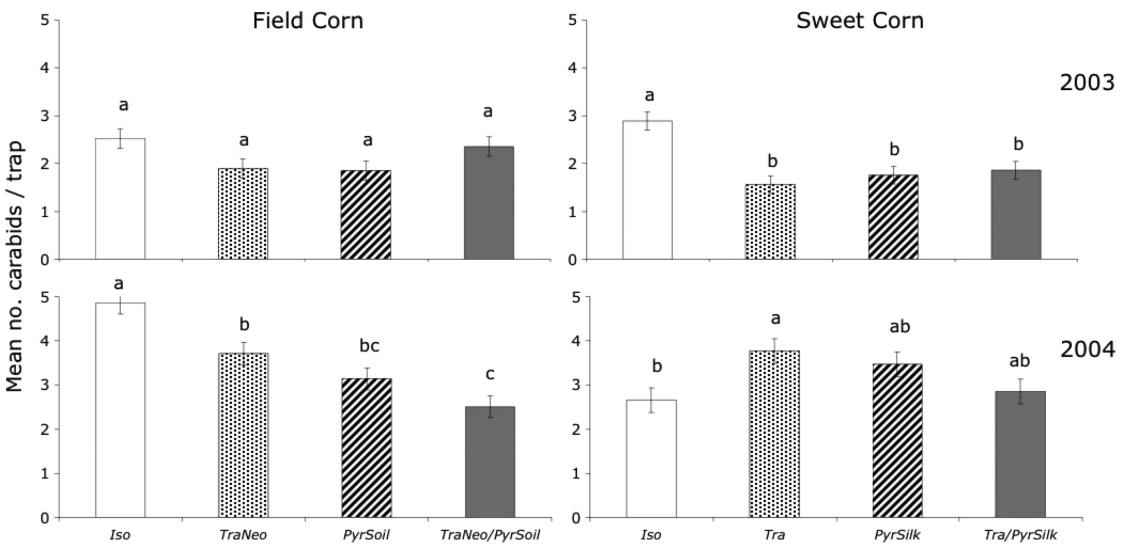


Fig. 2. A comparison of mean carabid activity densities (\pm SE) among three insect pest management strategies and a control (Iso) in field corn and sweet corn in 2003 and 2004.

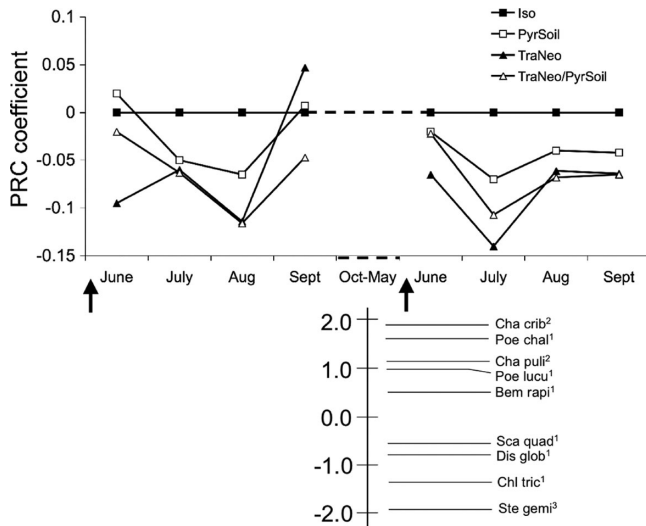


Fig. 3. PRC and taxon weights indicating response of nontarget coleopteran species to three insect pest management strategies in field corn over 2 yr in relation to a control (*Iso*; shown as horizontal 0.0 line on figure). Individual species weights are shown below the PRC. Arrows indicate when pyrethroid applications occurred. Species are denoted by the first three letters of the genus and first four letters of the species epithet (see Table 1 for full names). ¹Carabidae; ²Chrysomelidae; ³Nitidulidae.

corn after crop rotation. In second year field corn, the lowest activity densities were found in the treatment that contained both conventional pyrethroids and seed-based control methods.

Community Dynamics. Principal response curves showed a primarily negative response of the coleopteran communities in treatment plots relative to the control community in both field corn (Wilk's $\Delta = 0.546$; $F(34, 154) = 1.6$; $P = 0.029$; Fig. 3) and sweet corn (Wilk's $\Delta = 0.543$; $F(34, 154) = 1.62$; $P =$

0.026; Fig. 4). In field corn, the first measurements were taken after the first potential effect from either soil insecticides or neonicotinoid seed treatments. By July, the treatment communities deviated from the *Iso* community; all three treatment communities exhibited a steep decline in July and August, and then a return toward the *Iso* baseline in September. In 2004, a similar pattern emerged; however, the treatment communities were more uniform in their response pattern of decline and they did not re-

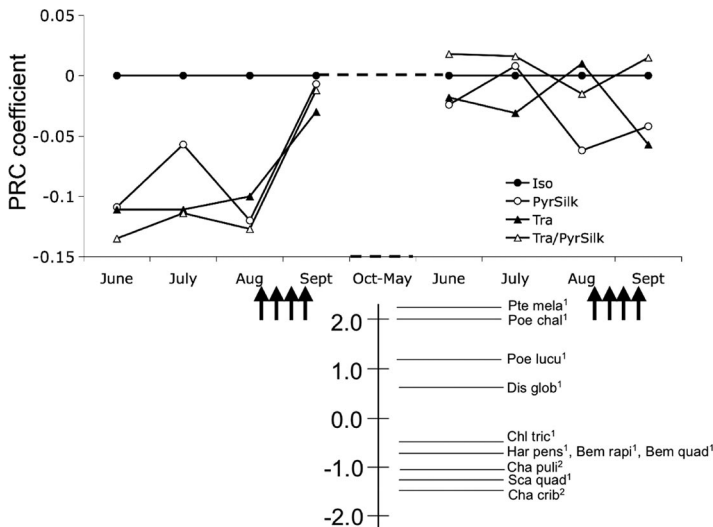


Fig. 4. PRC and taxon weights indicating response of nontarget coleopteran species to three insect pest management strategies in sweet corn over 2 yr in relation to a control (*Iso*; shown as horizontal 0.0 line on figure). Individual species weights are shown below the PRC. Arrows indicate when pyrethroid applications occurred. Species are denoted by the first three letters of the genus and first four letters of the species epithet (see Table 1 for full names). ¹Carabidae; ²Chrysomelidae.

bound completely to baseline level as in the previous year (Fig. 3).

For sweet corn in 2003, the treatment communities were distinctly separate from the control for June, July and August. In September, the communities were more similar in composition and activity density. In second year sweet corn, which had been rotated with snap beans, the treatment communities fluctuated above and below the control (Fig. 4).

Species weights indicated which species followed (species weight >0.5) or deviated (species weight <-0.5) from the principal response. In field corn, two chrysomelids (*Chaetocnema cribifrons* LeConte and *Chaetocnema pulicaria* Melsheimer) and three carabids [*Poecilus chalcites* (Say), *Poecilus lucublandus* (Say), and *Bembidion rapidum* (LeConte)] had species weights above 0.5, whereas three carabids (*Chaenius tricolor tricolor* Dejean, *Dyschirius globulosus* (Say), and *Scarites quadriceps* Chaudior) and one nitidulid [*Stelidota geminata* (Say)] had negative species weights below -0.5 .

In sweet corn, four carabids (*Pterostichus melanarius* Illiger, *P. chalcites*, *P. lucublandus*, and *D. globulosus*) had species weights above 0.5, whereas five carabids (*C. tricolor tricolor*, *Harpalus pensylvanicus* (DeGeer), *Bembidion quadrimaculatum oppositum* Say, *B. rapidum*, and *S. quadriceps*) and two chrysomelids (*C. cribifrons* and *C. pulicaria*) had species weights below -0.5 .

The redundancy analyses indicated that treatments did not have a significant effect on functional groups (Fig. 5). In field corn, the first axis was defined by the *Iso* treatment differentiating from the other treatments ($F = 2.51$; $P = 0.08$). Seed predators and herbivores were most strongly associated with the primary axis and the *Iso* treatment (Fig. 5A). In sweet corn, all functional groups had short vectors (Fig. 5B) indicating minimal effect sizes, and no significant pattern was detected ($F = 0.33$; $P = 0.82$).

Discussion

Coleopteran Community Response to Field Corn Treatments. Field corn was of primary interest in this study because of the presence of the coleopteran-specific Cry3Bb proteins in the transgenic treatment coupled with the neonicotinoid seed treatment, both of which could present direct effects on coleopterans. While many studies attempt to isolate the effect of an introduced transgene, we felt a realistic evaluation of the transgenic technology should include all insect pest management inputs that would be part of a transgenic maize system. Additionally, this study was preceded by laboratory bioassays examining the transgene and the seed treatments in isolation of one another (Mullin et al. 2005), where the seed treatment, and not the Cry3Bb protein, was found to be a significant mortality factor for carabids. Thus, ecologically relevant field assays were warranted.

The fact that field corn was not rotated throughout the 2-yr experiment made the second year field corn

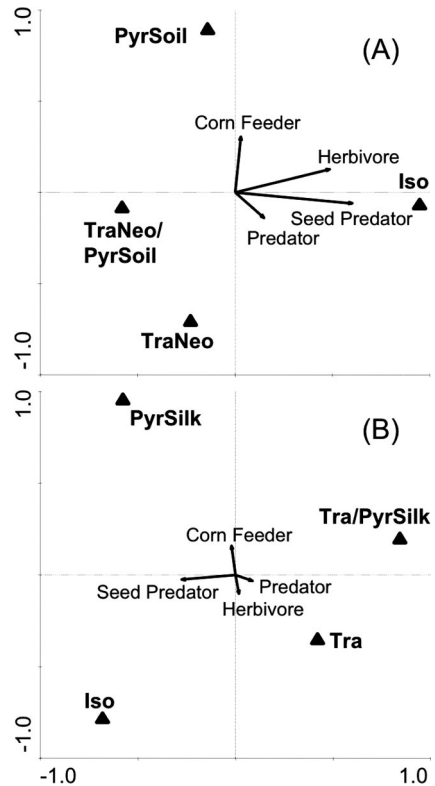


Fig. 5. Redundancy analysis bi-plots showing associations between coleopteran functional groups (vectors) and insect pest management strategies (triangles) in field corn (A) and sweet corn (B).

the most reliable indicator of treatment effects. Because of the large plot size (≈ 6 ha) required to conduct this experiment, the plots were initially situated over a previously diverse crop mosaic from the preceding year. In the second year, community legacy from previous land use was of less concern because field corn treatments remained in the same location for both years allowing coleopteran communities to establish.

We found no differences in interpolated Carabidae species richness among treatments, despite the use of pyrethroids, neonicotinoids, and Cry toxins. Notably, we recorded significantly higher levels of carabid species richness in adjacent noncrop habitat (Leslie 2007), indicating that our methods are sufficiently sensitive to detect differences in species richness and that the homogeneity and physical alteration of a crop field incurs the most dramatic effect to carabid species richness, regardless of within-field pest management practices. The heterogeneous habitat in Pennsylvania agroecosystems may be conducive to sustaining diversity in crop fields, as we were not able to detect differences in species richness among treatments in other cropping systems in the same region (see Leslie et al. 2007). Although broad-spectrum insecticides, such as organophosphates, can influence carabid diversity in field settings (Cárcamo et al. 1995, Epstein

et al. 2001), there is less evidence that pyrethroids impact the species richness of carabid assemblages (Holland et al. 2002). Despite the lack of differences in species richness among treatments, overall activity-density of carabids in the 2004 control treatment was significantly higher than the other treatments (Fig. 2). Therefore, by the second year of the study, carabid activity-density seems to be following a dose dependent response to the treatments; highest activity densities were found in the control and lowest activity densities were found in the treatment containing both conventional pyrethroids and the seed-based control methods.

The temporal dynamics of Coleoptera in the field corn suggested a negative response after insecticides (soil-applied or neonicotinoid seed treatments) were first introduced, followed by a recovery process later in the season (Fig. 3). This includes the seed-based technology treatment that did not receive any soil pyrethroids but did contain a neonicotinoid seed treatment in addition to Cry3Bb toxins. Based on our prior laboratory tests (Mullin et al. 2005), it is unlikely that Cry3Bb toxin presented any threat to nontarget coleopterans, whereas seed treatments were highly toxic to carabids. Bhatti et al. (2005) considered the effect of seed treatments and soil and foliar-applied insecticides on nontarget Coleoptera and, although results varied, they found significantly lower abundances of Carabidae and Nitidulidae in the seed treatment or soil-insecticide treatments as compared with a control in at least 1 yr of the experiment. This same study found relatively few differences between communities collected from *Bt* (Cry3Bb) and non-*Bt* field corn.

According to the PRC (Fig. 3) the treatment communities reconvene near the control community later in the season. This 'recovery' may represent a recolonization process and/or a gradual temporal shift in community composition because of the different life histories of the multiple species. Individual taxon weights indicated that two chrysomelids (*C. cribrifrons* and *C. pulicaria*) and three carabids (*B. rapidum*, *P. chalcites*, and *P. lucublandus*) closely followed the principal response. The corn flea beetle, *C. pulicaria*, may have been directly affected by feeding on corn with the systemic neonicotinoid. In the second year of the study, the herbivorous chrysomelids may also have been attracted to the control plots where weed abundances were higher (unpublished data, not quantified) because of corn knockdown by corn rootworm.

Conversely, the nitidulid, *S. geminata*, and three carabids, *S. quadriceps*, *D. globulosus*, and *C. tricolor tricolor*, responded in the opposite fashion. We expected *S. geminata*, to be higher in the control in the second year because it would likely be attracted to corn damage. However, pitfall traps measuring activity on the soil surface may not have accurately reflected such a trend. The carabid, *S. quadriceps*, is a large predator that emerges as an adult very early in the season before planting and thus may have avoided any treatment effects. Another carabid with a strong negative taxon weight, *C. tricolor tricolor*,

was much more abundant in the second year of the experiment and may have been filling the empty niche created by the decline of some species in the treated plots.

Using a redundancy analysis we examined how functional groups responded to the field corn treatments. Our species-level identification allowed us to group species based on their functional roles, with the added benefit of being able to include rare species (those excluded from the PRC) in the analysis. The RDA biplot (Fig. 5A) indicated that beetles, especially seed predators and herbivores, had higher activity densities in the control. Those species that feed directly on corn responded negatively to treatments containing the transgene and systemic neonicotinoid, whereas predators responded negatively to the soil-applied pyrethroids.

Coleopteran Community Response to Sweet Corn Treatments. Coleopteran communities in sweet corn did not exhibit any consistent trends across years. Additionally, no relationship between coleopteran functional groups and treatments were found. Using principal response curves, gradients in the sweet corn coleopteran communities were detectable in the first year of the study (Fig. 4), which may be a reflection of the communities defined by the diverse crop mosaic in the year preceding the experiment. The rotation of sweet corn in the second year seemed to remove the gradients found in the first year, possibly through species mixing as surmised in a similar study of rotated vegetable crops (Leslie et al. 2007). Vegetable crops, such as sweet corn, are commonly rotated in commercial production systems. The strong convergence in the second year may suggest a common influence of rotation behind a uniform crop (snap beans), as opposed to a rotation following a mosaic of crops as occurred in 2003.

No significant differences in carabid species richness were found among sweet corn treatments in either year. Although activity density was highest in the control in the first year, these trends did not persist in the second year when activity densities were highest in the transgenic treatment. It is unlikely that the reduction of carabids in the treated plots in the first year was because of transgenes or pyrethroids; the Cry1 transgene is directed at lepidopteran pests and the pyrethroids were not applied until late in the season.

In sweet corn, we used the same foliar insecticide (lambda-cyhalothrin) and similar application intervals as in Rose and Dively (2007), but our study focused on response by coleopterans caught on the soil surface, whereas their work considered a much broader range of taxa, including many in the vegetative portion of the crop. While they observed significant and strong community-level declines because of foliar insecticide treatments, these responses were much less from epigeal community members. Even among this epigeal community measured through pitfall traps, significant effects were fewer in Carabidae than other groups (mites, staphylinids), and where they occurred, popula-

tions increased in plots treated with foliar insecticides. Thus, our lack of an effect from foliar applications of pyrethroids is similar to what Rose and Dively (2007) report from similar taxa. Rose and Dively (2007) suggest three Carabidae species as potential bioindicators (*Pterostichus lucublandus*, *Chlaenius nemoralis*, and *Harpalus pensylvanicus*) in sweet corn. Our observed responses included two of these species (*P. lucublandus* and *H. pensylvanicus*), and may warrant further population level assessment.

In conclusion, species-level identifications provide the highest level of ecological resolution in ecotoxicological studies, and functionally diverse subsets of communities can be used to augment studies using taxonomic surrogates (e.g., family level identifications) that address a larger numbers of specimens. Although initial investment of time and monetary resources for species-level identifications can be substantial, we found that subsequent years of study become easier because of the development of voucher collections and increased taxonomic expertise or established taxonomic collaborations.

This study indicates that some members of the nontarget coleopteran community may respond to seed-based technology management practices in maize. This was more evident in the second year of nonrotated field corn in which communities were able to establish and were exposed to 2 yr of the same treatments in the same location. Although longer-term studies are needed, early indications are that some nontarget herbivores may be affected when Cry proteins are coupled with systemic neonicotinoids as currently found on transgenic field corn seed, or by pyrethroids applied to the soil at planting. Results for carabids and nitidulids varied between species, however, principal response curves indicated a tendency for higher overall activity-densities in the untreated control. Despite some species-specific effects, impacts on ecosystem function are likely minimal. When we examined the response of broader functional groups to conventional and seed-based insect management strategies, no significant effects were found. It is also important to note that the control treatment did not necessarily represent a viable management option. Knockdown because of corn rootworm was evident in the control treatment of second year field corn and small shifts in microclimate and weed abundance may have also contributed to differences in the coleopteran communities between the control and the other treatments.

Seed-based and conventional management effects on nontarget coleopterans were similar, as both showed a transitory deviation from the untreated control. Nontarget effects in maize using seed-based technologies were likely because of the presence of the neonicotinoid seed treatment associated with the transgenic seed, because there is no evidence that Cry proteins are directly affecting these nontarget organisms, although nontarget chrysomelids are known to uptake Cry toxins from transgenic maize agroecosystems (Harwood et al.

2005). There is the possibility of prey-mediated effects on nontarget species because of the introduction of transgenes in an agroecosystem; however, effects are thought to be minimal as generalist predators may exhibit prey switching or increases in food intake to account for reduction in prey quality (Lundgren et al. 2009). This work in maize, and in vegetable systems (Leslie et al. 2007), suggests coleopteran community-level effects may be more apparent in nonrotated systems. The results of the laboratory (Mullin et al. 2005) and field-based trials suggest that further longer-term studies are warranted for systems using coupled transgenic-neonicotinoid technology where lack of crop rotation is common.

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