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EPIGEAL INSECT DIVERSITY AND DYNAMICS IN AGROECOSYSTEMS

ADOPTING TRANSGENIC CROPS

A Thesis in

Entomology

by

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ABSTRACT

Using data from five years of field experiments, we assessed the influence of insecticidal transgenic crop technologies on non-target epigeal insect diversity in both maize and diversified vegetable agroecosystems. Concurrently, we considered the relative influence of crop species, crop rotations, and proximity to forested field margins on these insect communities. Our approach differed from other transgenic non-target studies by emphasizing species-level identification of a functionally diverse subset of the epigeal insect community, notably Coleoptera, with extensive analysis of Carabidae, to maximize the biological and functional resolution of the data. We examined population dynamics as well as community response using current statistical approaches in the fields of biodiversity sciences and community ecology.

Insecticide inputs were lower in transgenic crops due to the efficient control of important pest taxa, which served to sustain epigeal biodiversity. In maize agroecosystems, no differences were found between the coleopteran communities in conventional and transgenic insect control practices where transgenic field corn contained a neonicotinoid seed treatment. Both management tactics negatively influenced coleopteran communities compared to a control. Dominant members of the carabid community exhibited temporal partitioning of resources. One species, *Harpalus pensylvanicus*, consistently exhibited higher activity-densities in the control fields, and was identified as a possible bio-indicator. Despite management influences, differences between crop species and the effect of crop rotations was often sufficient to mask any treatment effects.

Carabid communities were significantly different between maize and a forested field margin, and shifts in community structure along a transect were most rapid at the grassy margin between the two habitats. Carabidae communities were more diverse in the forest edge, where plant diversity was also highest. Despite strong habitat associations, most abundant carabids were found in both forest and maize. Differences between carabid communities in maize fields at varying distances from a forested margin suggested landscape level influences. In conclusion, the spatial and temporal heterogeneity of agroecosystems in the northeastern U.S. seemed to promote on-farm

insect diversity, although non-target effects from transgenics coupled with neonicotinoid seed treatments and conventional insect control methods were discernible from a control.

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Chapter 1

Introduction

Ever since the beginning of the environmental movement in the 1960's, largely spurred by the publication of Rachel Carson's *Silent Spring*, there have been efforts to understand and quantify the impact of anthropocentric activities on human and environmental health. In the following decade, the field of ecotoxicology originated and terms such as "biodiversity" were coined and are now commonplace. Agriculture, which for the last 50 years has been largely dependent on synthetic chemical pesticides for pest control, has been an important area of focus regarding environmental impacts. *Silent Spring* resulted in the removal of DDT from U.S. agriculture, and the Food Quality and Protection Act (FQPA) of 1996 initiated further restrictions resulting in the discontinuation of many pesticide chemistries, primarily in the organochlorine, organophosphate and carbamate families. Alternatives such as reduced-risk and biorational pesticides, biological control agents, transgenic crops, and organic practices have since been introduced as part of an integrated pest management (IPM) approach. Despite their perceived role as lower-risk alternatives, stringent risk assessment procedures are required for all new pest management tactics – even biological control agents can become serious pests, without proper knowledge of their ecology.

Among the farm management inputs that may directly or indirectly affect non-target organisms and their diversity, few have received as much attention as transgenic, or genetically engineered (GE), crops. GE crops were commercially introduced in the mid 1990's and include herbicide-tolerant, virus-resistant, and insect-resistant varieties to aid in control of agricultural pests. Although ecological concerns regarding non-target

effects, gene flow, and pest resistance are legitimate, public fears have been amplified and more vociferously proclaimed for transgenic crops than any other contemporary pest management strategy. Part of this can be explained by early reports that transgenic corn pollen may be toxic to monarch butterflies (Losey et al. 1999) – a charismatic insect that resonated with the general public and for which news spread rapidly through media sources and environmental groups. Fear of the unknown regarding genetic manipulation of food sources and impacts to human health also played a role, and terms such as “frankenfood” were common in news reports and publications. Additionally, ethical concerns have been raised about the role humans should play in genetic engineering. Despite a long history of genetically *modifying* crops through plant breeding, genetic *engineering* of crops (and other organisms) by combining genetic material from non-breeding organisms has led to heated ethical debate.

This thesis examines one of the main concerns regarding transgenic crops: non-target effects. The primary focus is on insecticidal transgenic crops and their impacts on insect diversity and dynamics. Currently, insecticidal transgenic crops are often referred to as *Bt* crops as they contain transgenes from the soil bacterium, *Bacillus thuringiensis*, that encode for insecticidal crystal (Cry) proteins, also known as δ -endotoxins. These Cry proteins are specific to certain pest taxa and have been primarily directed at lepidopteran (Cry1-based proteins) and coleopteran (Cry3-based proteins) pests. Over the last decade, the adoption of *Bt* crops (especially cotton and maize) in U.S. agriculture has risen steadily. The introduction of *Bt* crops has been accompanied with concern for direct or indirect negative effects to non-target insects (Angle 1994, Groot and Dicke

2002), especially those in the same insect order for which the Cry proteins are directed or for important biological control organisms found in agricultural fields.

Numerous studies in both the field and the laboratory have examined such effects for a variety of invertebrates, including (among others): butterflies (Losey et al. 1999, Wraight et al. 2000), lacewings (Hilbeck et al. 1998), ladybird beetles (Al-Deeb and Wilde 2003, Zhu et al. 2006), and ground-dwelling beetles (Duan et al. 2004, Bhatti et al. 2005, Mullin et al. 2006). To date, most evidence suggests few non-target effects (summarized by Romeis et al. 2006), except in instances where the abundance of a specialist predator of the target pest taxa is reduced due to lack of prey (i.e., Pilcher et al. 2005). Additionally, the need to address sub-lethal effects or gradual shifts in non-target communities with long-term ecological studies has been highlighted (Shelton et al. 2002).

Despite the lack of documented non-target effects, there remains a need for non-target studies assessing effects to communities and diversity with species-level taxonomic resolution. Field studies examining communities often resort to family-level taxonomic resolution (e.g., Bhatti et al. 2005, Rose and Dively 2007), while those with species-level taxonomic resolution are primarily population studies examining one or a few species (e.g., Lopez et al. 2006). When examining community-level response, species identification provides the greatest and most accurate ecological inference. Additionally, the use of taxonomic surrogates in ecotoxicological experiments can result in erroneous conclusions; higher taxa of insects often contain numerous species representing different sizes and functional roles that may respond differently to the same environmental conditions (Danks 1996). Species identification also allows for diversity measurements

such as species richness and evenness, and provides baseline species data for the sampled area at a unique point in time.

This thesis consists of a series of field studies examining the adoption of transgenic crops in agricultural settings representative of northeastern U.S. agroecosystems, and compares their impact on non-target insect communities to conventional insect control practices. Agriculture in the northeastern U.S. consists of numerous small, often diversified, farms embedded in a heterogeneous landscape. Therefore, our studied cropping systems consisted of both vegetable and field crops, often in small-farm cropping mosaics. Additionally, one study also considered the habitat adjacent to crop fields by examining the relative importance of field margins on insect diversity within the crop. These studies were characterized by a strong taxonomic investment (i.e., species-level identification), temporal considerations (i.e., sampling on numerous dates throughout growing season), and rigorous statistical analyses for measuring community patterns and dynamics.

We focused on epigeal, or soil-surface dwelling, invertebrates as the non-target community. Epigeal insects are often included in ecological analyses due to their contribution to ecosystem functions [e.g., regulation of invertebrate herbivores (Asteraki 1993, Clark et al. 1994) and weed seed banks (Hartke et al. 1998; Tooley and Brust 2002)], ease of collection using pitfall traps, high capture rate of species and individuals, which can increase the power of statistical analyses, and their sensitivity to variation in agricultural management (Duelli et al. 1999). In all the studies, extensive consideration was given to Carabidae as this family was consistently the most abundant and speciose group collected, and we were able to identify all specimens to species. Carabids are

generally considered beneficial to agriculture as most are voracious feeders and comprise numerous functional groups including generalist invertebrate predators, specialist predators (i.e., snail feeders), and weed seed predators (Toft and Bilde 2002, Westerman et al. 2003).

A brief synopsis of the three experiments and their contribution to the scientific community are as follows:

Study I. The first study (Chapter 2) used a farm systems approach to examine the response of epigeal insect communities in diversified vegetable farms utilizing transgenes to those relying on conventional management practices. To date, no study has examined transgenes in a diversified farm setting typical of a northeastern U.S. farm. The diversified vegetable plots contained sweet corn, potatoes, and acorn squash, which were rotated in the second year of the two-year study. Transgenic and conventional management plots were both managed according to common IPM practices (i.e., scouting and spraying for pests exceeding thresholds, crop rotations), thus accounting for the farm systems approach. Therefore, this study quantified differences in insecticide inputs between transgenic and conventional diversified vegetable farms, and looked for cascading effects on insect biodiversity. Additionally, we examined the relative influence of pest management inputs versus the influence of crop type and crop rotations on the epigeal communities.

Study II. The second study (Chapters 3 and 4) focused exclusively on maize crops (sweet corn and field corn). The experimental design examined four treatments in each type of maize: 1) non-transgenic maize with no insecticides, i.e., control, 2) non-transgenic maize with conventional insecticides, 3) transgenic maize, and 4) transgenic

maize with conventional insecticides. Transgenic field corn was of primary concern as it represented a new coleopteran-specific Cry protein (Cry3Bb) and also came coupled with a neonicotinoid seed treatment not found on the near isoline (non-transgenic) seed. We introduced the term “transgenic technology” to represent the use of these coupled technologies in transgenics. In Chapter 3 we identify the most abundant Carabidae and examined population-level response. Chapter 4 looks at the community dynamics of three coleopteran families (Carabidae, Chrysomelidae, and Nitidulidae) in which all specimens were identified to species and represented a diversity of functional roles.

Study III. The final study (Chapter 5) was designed to investigate the influence of field margin habitat on Carabidae diversity within crop fields. Traps were deployed in transects spanning field corn, a mowed grassy margin, and an adjacent forest edge. Using the transect design, we examined shifts in the carabid community structure across habitats and specifically at ecotones. We introduce a novel adaptation of principal response curves (PRC) to examine community dynamics over a spatial gradient. Within the field corn we used a paired design to compare Carabidae communities between transgenic and conventionally managed field corn. Additionally, we address landscape-level influences on insect communities by comparing the carabid communities found in the forest-edge corn, to the carabid communities found in the previous studies (Chapters 2-4), which were embedded in an agricultural landscape.

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Chapter 2

Transgenes Sustain Epigeal Insect Biodiversity in Diversified Vegetable Farm Systems

ABSTRACT

Many ecological studies have focused on the effects of transgenes in field crops, but few have considered multiple transgenes in diversified vegetable systems. We compared the epigeal, or soil surface-dwelling, communities of Coleoptera and Formicidae between transgenic and isoline vegetable systems consisting of sweet corn, potato, and acorn squash, with transgenic cultivars expressing Cry1(A)b, Cry3, or viral coat proteins. Vegetables were grown in replicated split plots over two years with integrated pest management standards defining insecticide use patterns. Over 77.6 % of 11,925 insects from 1,512 pitfall traps were identified to species, and activity-density was used to compare dominance distribution, species richness, and community composition. Measures of epigeal biodiversity were always equal in transgenic vegetables, which required fewer insecticide applications than their near isolines. There were no differences in species richness between transgenic and isoline treatments at the farm system and individual crop level. Dominance distributions were also similar between transgenic and isoline farming systems. Crop type, and not genotype, had a significant influence on Carabidae and Staphylinidae community composition in the first year, but there were no treatment effects in the second year, possibly due to homogenizing effects of crop rotations. The heterogeneity of crops and rotations in diversified vegetable farms seems to aid in preserving epigeal biodiversity, which may be supplemented by reductions in insecticide use associated with transgenic cultivars.

INTRODUCTION

Agriculture, which covers roughly one-half of usable land on the planet, inevitably affects biodiversity, and its expansion and/or intensification could further impact biodiversity and ecosystem services (Stoate et al. 2001; Tilman et al. 2001; Tilman et al. 2002). This has become a concern reflected in international policy; in 2002, 190 countries committed to the goals of the Convention on Biological Diversity which proposed to reduce the rate of biodiversity loss by the year 2010 (Balmford et al. 2005). Managing agricultural pests and maximizing crop yields, while simultaneously minimizing the effects to non-target organisms, has become an important challenge in modern farm management (Green et al. 2005).

The influence of pest management on non-target communities has drawn much attention with the increased development and use of transgenic crops. Many have looked specifically at monocultures of field crops using experimental designs intended to isolate the effect of introduced transgenes (e.g., Sisterson et al. 2004; Dively 2005; Whitehouse et al. 2005). Some, typically dealing with herbicide tolerant crops (but also see Cattaneo et al. 2006 for a recent example including insect-resistant cotton), have used a farm systems approach where differences in chemical inputs as a result of the introduction of transgenics are inherently expressed as part of the experimental design (e.g., Squire et al. 2003). However, no studies have considered diversified vegetable farm systems, which include different insecticide use patterns tied to integrated pest management (IPM) practices than are common in modern agriculture. In order to understand the effect of transgenic crops on biodiversity, more studies are needed that incorporate pest management changes that occur when using transgenic crops (e.g., changes in pesticide

use patterns), while still representing common farm system practices (e.g., crop rotations).

Invertebrates comprise a large part of the biodiversity in agroecosystems and the adoption of transgenic crops could influence invertebrate diversity (Angle 1994; Groot and Dicke 2002). Indirect effects on nontargets, including reduction of bees, butterflies and weed seed eating carabids, were present with adoption of certain herbicide tolerant field crops in Great Britain (Brooks et al. 2003, Haughton et al. 2003). In contrast, eleven large ecotoxicology experiments showed little or no detrimental effects of insecticidal transgenic cultivars of field crops on insect diversity and abundance (Naranjo et al. 2005). Riddick et al. (2000) found no differences in coccinellid, formicid or epigeal carabid populations in transgenic and non-transgenic potato farming systems that included foliar insecticide treatments timed with IPM thresholds. The epigeal, or soil surface-dwelling, invertebrate community is often included in ecological analyses due to their contribution to ecosystem functions [e.g., regulation of invertebrate herbivores (Asteraki 1993, Clark et al. 1994) and weed seed banks (Hartke et al. 1998; Tooley and Brust 2002)], ease of collection, and high capture rate of species and individuals, which can increase the power of statistical analyses (Duelli et al. 1999). Additionally, epigeal taxa can be good bio-indicators of varying habitat features or farm management systems (e.g., Carabidae: Carcamo et al. 1995, Ellsbury et al. 1998, Holland and Luff 2000, Thorbek and Bilde 2004; Staphylinidae: Lassau et al. 2005; Formicidae: Andersen and Majer 2004).

At least three transgenic vegetable crops with insecticidal proteins, or with gene constructs that influence insect-transmitted plant pathogens, have been commercially

considered or are being introduced into northeastern U.S. agroecosystems. These include: (1) sweet corn expressing the Cry1(A)b protein that protects against several lepidopterans, (2) potatoes that express the Cry3 protein aimed primarily against *Leptinotarsa decemlineata*, and (3) acorn squash (*Cucurbita* spp.) that express viral coat proteins that protect against several aphid-transmitted viruses. In diversified fresh-market farms in the northeastern U.S., all three crops often occur on the same farm, in agroecosystems comprised of a complex mosaic of small fields or even multiple crops within a field. Although the transgenic potato cultivars were recently removed from commercial application, they could be re-introduced; the acorn squash cultivars are commercially available and commonly grown in some areas, and the sweet corn cultivars appear to be slowly gaining in adoption rates. These complex and constantly changing agroecosystems contain a greater variety of food resources and refuges, which should allow organisms to partition resources both temporally and spatially, permitting a larger complex of species to exist (Root 1973; Anderson 2000; Landis et al. 2000).

Here, we compare the diversity of soil surface-dwelling beetles and ants within diversified transgenic and non-transgenic vegetable farming systems that followed current IPM standards for insecticide use patterns. We use species richness and dominance distribution comparisons and direct gradient ordination analyses to examine the influence of transgenic vegetable cultivars on epigeal beetle and ant diversity. We hypothesized that transgenic and non-transgenic farm systems would require different pest management inputs, which would result in differences in biodiversity. Further, we expected that these differences would depend upon crop type.

METHODS

Experimental design. We grew vegetables in a split plot design at the Russel E. Larson Experimental Farm in Rock Springs, Pennsylvania, in 2001 and 2002. Two ‘genotype’ main plots—consisting of all transgenic or all isoline (identical line without transgenes) cultivars—were replicated three times. In 2001, each main plot was split among three crops: sweet corn, acorn squash, and potatoes. In 2002, each main plot was split among two crops: sweet corn and potatoes (due to crop failure, acorn squash was not grown). Cultivars (isoline and transgenic, respectively) consisted of 'Jackpot' and 'BC 0801' expressing the Cry1Ab δ endotoxin for sweet corn, 'Taybelle' and 'Taybelle PM' expressing plant viral coat proteins for acorn squash, and 'Superior' and 'New Leaf Superior' expressing the Cry 3A δ endotoxin for potatoes.

Main plots were 61 x 30.5 m (0.186 hectares) and subplots were 20.3 X 30.5 m (0.062 hectares) for a total of 1.116 hectares (2.76 acres) in vegetable crops; in addition, we established mowed alfalfa/grass borders around all main plots, resulting in a 1.22 ha (3 acre) experimental field with drip irrigation per year. The experiment was planted in spring 2001 following an alfalfa crop. In 2002, the sweet corn was rotated after potatoes, and potatoes after the acorn squash, while keeping the transgenic or isoline cultivars within their respective ‘genotype’ mainplots. The sweet corn and potatoes were planted with a 0.30 m seed spacing totaling 100 plants/row or 2,600 plants/corn subplot (0.76 m row spacing) and 1,900 plants/potato subplot (0.91 m row spacing). The acorn squash was planted in plastic mulch, was drip-irrigated, and was spaced at 1.22 m for a total of 25 plants/row or 275 plants/subplot (1.83 m row spacing). Planting dates were May 8

(potatoes), May 31 (sweet corn), and June 6 (acorn squash) in 2001, and May 13 (potatoes) and May 28 (sweet corn) in 2002.

We monitored for pests within subplots twice a week by scouting 10 plants/subplot in all crops, and using sweep nets in potatoes. We also established and serviced traps baited with pheromones (Hercon Environmental, Emigsville, PA) to monitor corn earworm, European corn borer and fall armyworm. In both transgenic and isoline mainplots, insecticides were applied when the average pest population among all replicates of a main-plot treatment for that crop reached predetermined thresholds described in Foster & Flood (1995). Based on standard potato production practices, imidacloprid was applied at-planting (without pest monitoring) in the isoline plots. All insecticides were applied at the midpoint of the labeled rate range. Applications of fungicides (in potatoes) and herbicides (in all crops) did not vary among treatments, and are described in Hoheisel (2002).

Insect collection and identification. In both 2001 and 2002, we placed six pitfall traps in each subplot. The pitfall traps, 14.1 cm deep with 10.9 cm inside diameter, were inserted flush with the ground and contained small collection cups (5.5 cm deep, 8.2 cm inside diameter) filled with ethylene glycol. Inverted funnels, made from 2-liter soda bottles, fit inside the rim of the plastic container. The funnels and their placement within the pitfall trap provided a barrier between the collection container and small mammals (Morrill et al. 1990). Between trapping periods, the traps were closed with a plastic lid.

Three pitfall traps per subplot were open at any given time. In 2001, we opened three traps early in each week for 24-hours, and the other three pitfall traps later in the

same week also for 24-hours. Due to decreased labor availability in 2002, three pitfall traps were opened for 48 hours once a week, and were alternated weekly with the other three traps. For both years, all data were recorded as number / 48 hours / week.

Therefore, for each week we recorded data from 54 traps (3 traps * 3 crops * 6 main plots). The collection period (late June to late August) lasted for 8 weeks in 2001, and 9 weeks in 2002.

We emptied pitfall traps onto a 420 micron (0.004 cm) sieve and sorted out all ants (only in 2001) and beetles. Insects were preserved in 70% ethanol. R. Davidson and R. Andow (Carnegie Museum of Natural History, Pittsburgh, PA) identified or confirmed identifications of Buprestidae, Cerambycidae, Carabidae and Cicinidelidae. R. Hoebeke of Cornell University identified most of the 2001 Staphylinidae. All additional taxonomic work was done by D. Biddinger (Coleoptera) and G. Hoheisel (Formicidae) of the Pennsylvania State University. Voucher specimens are currently being held in the Fleischer Lab, Department of Entomology, Pennsylvania State University.

Data analysis. We compared epigeal biodiversity between transgenic and isoline cultivars at the farm system level, where all crops were combined for transgenic or isoline cultivars, and individual crop level using multivariate analyses and measures of species richness and evenness to investigate effects of treatment variables (i.e., crop, genotype and crop-genotype interactions) on community composition. We estimated species richness with rarefaction curves (Gotelli and Colwell 2001) using EstimateS 7.5 (Colwell 2005). Samples were randomly re-ordered 50 times and standardized to the number of individuals caught. Rarefaction curves were developed for all specimens, and for Carabidae separately, since all carabids were identified to species level. Significance

was determined by non-overlapping confidence intervals. We examined the dominance distribution of the Carabidae and Staphylinidae communities, the two most abundant families, between years and treatments with rank abundance curves. Percent of total abundance was used to determine the most dominant species, those representing > 1% of total abundance.

We performed redundancy analyses (RDA) using CANOCO 4.5 (ter Braak and Šmilauer 2002) to determine associations between species and treatment variables (crop, genotype and crop-genotype interactions) for Carabidae and Staphylinidae. RDA is a form of direct gradient analysis, or constrained ordination, where the ordination of the species data is constrained by the axes determined by the treatment variables (Lepš and Šmilauer 2003). Since the experimental design had two levels of variability (genotype = whole plot; crop type = split plot) we used a split-plot restriction in our analysis. Treatment variables were assessed using Monte Carlo simulations with 999 iterations and forward stepwise selection. Abundance data were Hellinger-transformed (Legendre and Gallagher 2001), centered and standardized. Species scores were divided by the standard deviation after axes extraction. Bi-plot diagrams of the ordination results were generated using CanoDraw (ter Braak and Šmilauer 2002).

RESULTS

Insecticide inputs. Transgenic and isoline crops received different insecticide treatments based on commonly used preventative measures, such as at-planting applications, and pest threshold scouting. The isoline cultivars received 11 insecticide

applications, whereas the transgenic cultivars received 8 applications, primarily due to the efficiency of the transgenic cultivars at protecting against major pests. Insecticide use information is detailed in Table 1.

Insect collection and taxonomic resolution. A total of 11,925 beetle and ant specimens were collected in 1,512 pitfall traps over both years. Activity-density rates were fairly consistent between years as 864 traps accumulated 6,750 specimens (7.8 specimens / trap / 48 hr) in 2001, and 648 traps accumulated 5,175 specimens (7.9 specimens / trap / 48 hr) in 2002. Carabidae and Staphylinidae represented more than half of all specimens collected with 4,261 and 2,677 specimens, respectively. All other Coleoptera totaled 2,885 specimens and Formicidae (collected only in 2001) totaled 2,102 specimens.

Coleoptera consisted of a diverse array of species belonging to 32 families (Table 2; Table 3 Carabidae; Table 4 Staphylinidae). 77.6% of specimens were identified to the species / morphospecies level, including all Carabidae (Table 3) which consisted of 47 species. 94% of the Staphylinidae were identified to species or genus level (Table 4), representing at least 30 species. Formicidae consisted of seven genera, which also are included in Table 2.

Species richness. There were no significant differences in rarefied species richness estimates of all taxa between isoline and transgenic cultivars at the farm system or crop level in either year (Fig. 1, 2). Although the transgenic treatments exhibited higher species richness in all instances except sweet corn in 2002, the 95% confidence intervals were always overlapping (not shown). There was a trend of lower species richness in 2002, however comparison of all taxa between years is not possible due to the

removal of acorn squash from the fields and because no formicid taxa were included in the 2002 analyses.

Carabidae had no differences in rarefied species richness estimates at the farm system or crop level for either year (Fig. 3, 4). As in the all taxa analyses, species richness estimates were always higher in the transgenic treatments except for corn in 2002, but the 95% confidence intervals were overlapping in all instances (not shown). Carabidae species richness estimates were consistently lower in 2002. At the farm system level this may be due to the removal of acorn squash from the experiment, however direct comparisons can be made for sweet corn and potato at the crop level. In sweet corn, rarefied estimates at 300 individuals resulted in 17-23 species for 2001, but only 13-14 species in 2002 (Figure 4). For potato, rarefied estimates at 300 individuals resulted in 16-19 species for 2001 and 15-16 species in 2002.

Dominance distribution. Dominance distributions of the Carabidae community were highly skewed due to the extremely high abundance of a single species, *Pterostichus melanarius* (Illiger), which accounted for 73.5% of all carabids. Out of 47 carabid species, only 7 species accounted for > 1% of the total abundance at the farm systems-level (for both years combined), while all other carabid species combined accounted for 8.1% of total abundance. In addition to *P. melanarius*, five species were among the most abundant in both years: *Poecilus chalcites* (Say), *Harpalus affinis* (Shrank), *Harpalus pensylvanicus* (DeGeer), *Anisodactylus sanctaecrucis* Fabricius, and *Poecilus lucublandus* (Say). The order of dominance varied between years, however *P. melanarius* was always the most dominant species (Fig. 5).

Staphylinidae displayed a skewed dominance distribution as well, although the most abundant species was not consistent between years (Fig. 6). In 2001, *Dinaraea angustula* (Gyllenhal) was the most dominant species and accounted for 55% of the total abundance. In 2002, the Staphylinidae community exhibited a more even distribution, and *Hoplandria lateralis* (Melsheimer) was the most dominant species, representing 38.4% of the total abundance. In addition to *D. angustula* and *H. lateralis*, *Strigota ambigua* (Erichson) was among the three most abundant species in both years. The rank abundance of the remaining species was similar in both years.

Genotype and crop influence on community composition. A redundancy analysis (RDA) of the 2001 Carabidae data revealed that there were differences in community composition associated with crop type (Fig. 7a). Potato significantly differentiated from the other crops ($F = 3.75$, $P = 0.002$), and explained 21.4% of the unconstrained species variation and 60.2% of the constrained species variation ($\lambda_1 = 0.21$). Of the most influential species, *P. melanarius* was highly associated with potato. The remaining species were more highly associated with sweet corn and acorn squash. In 2001, genotype (isoline versus transgenic) did not have significant effects ($F = 1.19$, $P = 0.808$) on Carabidae community composition, although *Agonum muelleri* (Herbst) and *Elaphropus anceps* (LeConte) were collected more often in transgenic plots while *A. sanctaerucis* and *P. lucublandus* were found more often in isoline plots (Fig. 7a). No significant treatment effects due to crop ($F = 1.12$, $P = 0.096$) or genotype ($F = 0.53$, $P = 0.902$) on Carabidae community composition were found in 2002 (not shown). A redundancy analysis (RDA) of the Staphylinidae data yielded results that were similar to that for Carabidae. Crop type had a significant influence on Staphylinidae community

composition (Fig. 7b) in 2001. Potato significantly differentiated from the other crops ($F = 4.72$, $P = 0.002$) and explained 24% of the unconstrained species variation and 52.6% of the constrained species variation ($\lambda_1 = 0.24$). Seven of the nine most influential species had higher activity densities in transgenic management, however genotype ($F = 2.31$, $P = 0.092$) did not have a significant effect on Staphylinidae community composition. In 2002, crop ($F = 2.19$, $P = 0.058$) and genotype ($F = 0.98$, $P = 0.69$) had no significant effect on Staphylinidae community composition (not shown).

DISCUSSION

Insecticidal transgenic crops have the ability to reduce the number of insecticide applications in transgenic vs. conventional farm systems (Lynch et al. 1999, Qaim et al. 2003, Hutchison et al. 2004). Many studies have documented negative effects of insecticides on non-target invertebrates, including decreases in species richness or abundance of important predators, such as carabids (e.g., Los & Allen 1983, Teodorescu & Cogalniceanu 2005). Duan et al. (2004) compared transgenic and non-transgenic potato management on non-target communities and found a significant decrease in spider abundance due to permethrin but not transgenic management. In lab trials, Mullin et al. (2006) found no detrimental effect of *Bt* pollen fed to carabids, but documented high levels of mortality due to neonicotinoid seed treatments. These trends suggest a possible difference in biodiversity between transgenic and non-transgenic management regimes as typified by a dose or frequency dependent response. Due to a high degree of target specificity and possibility for insecticide reductions, certain *Bt*-transgenic crops may be

important in sustaining biodiversity on agricultural lands and an important component of integrated pest management systems utilizing biological control (Musser & Shelton 2003, Romeis et al. 2006).

In this study, transgenic and isoline diversified vegetable crops, consisting of sweet corn, potato and acorn squash, required different insecticide inputs based upon IPM pest thresholds and normal management procedures. Over 2 years, transgenic crops received fewer insecticide applications than the isoline crops (isoline = 11; transgenic = 8). This difference was primarily due to the ability of the transgenic cultivars to effectively control the target pests, in that transgenic sweet corn and transgenic potatoes required no insecticide applications for lepidopteran pests or Colorado potato beetle, respectively. Conversely, the isoline cultivars received insecticide inputs in the form of a preventative at-planting neonicotinoid application in potatoes, or responsive pyrethroid applications during reproductive stage of sweet corn. There were instances where the greater selectivity of the transgenic system increased the number of foliar insecticide applications. For example, transgenic potatoes did not control *Empoasca fabae* (Harris), whereas imidacloprid at-planting did early in the season, resulting in more foliar sprays directed at *E. fabae* in transgenic than isoline potatoes.

Despite differences in insecticide inputs, there were no detectable differences in species richness (Figs. 1-4) between transgenic and isoline treatments, whereas crop type had the only significant effect on biodiversity by altering community composition (Fig. 7). In almost all cases, transgenic treatments had higher species richness estimates, however these differences were not significantly different (Figs. 1-4). There was evidence of a decrease in species richness from 2001 to 2002 for carabids in sweet corn

and potatoes (Fig. 4). This may be a result of the increase in management intensity on our study site, such as soil tillage and insecticide applications, as compared to the previous conditions of the uniform alfalfa stand in 2000. Interestingly, the decrease in carabid species richness from 2001 to 2002 appeared to be due to the loss of rare species, since the dominant species composition was similar between years (Fig. 5).

Multivariate analyses of community composition indicated that crop type had the largest effect on Carabidae and Staphylinidae communities in 2001 (Fig. 7). The diversified farm plots were preceded by a uniform alfalfa crop in 2001. Therefore, epigeal beetle communities may have been defined by the different crops, which represent differences in vegetative architecture, soil manipulation and pest management practices. These combined characteristics may have created habitat niches for various species as seen in Figure 7. No treatment variables explained community composition in 2002. By 2002, crop rotations may have confounded any distinct community assemblages that were developing in 2001. Thus, crop rotation may act as a mechanism for species mixing in diversified, small-scale agroecosystems.

The carabid community was defined by species positively or negatively associating with potato (Fig. 7a). *P. melanarius* was the only species that seemed to prefer potato plots. Potato could be considered the most highly “disturbed” habitat due to the intense cultivation (i.e., hilling) of the soil as well as insecticide use in the isoline plots. *P. melanarius* thrives in highly disturbed habitats and may have found an interspecific competitive advantage in this crop type. The other influential carabid species were common in acorn squash and sweet corn. Acorn squash plots and early season sweet corn plots contained the most open environment, allowing weed invasion

(not quantified) in which *A. sanctaecrucis* and *H. pensylvanicus*, weed seed predators, may have found an important food resource. *Poecilus* spp. may have been attracted to the open foraging environments (Larochelle and Larivière 2003) of sweet corn and acorn squash, which could account for their higher abundances in these crops.

Species-level community analyses of Staphylinidae are rare. Our work showed at least 29 epigeal species existing in northeastern agroecosystems. A redundancy analysis of the staphylinid community indicated that species were separating out by those associated with potato and those associated with sweet corn and acorn squash. Of the most abundant species, *H. lateralis* and *S. ambigua* were found more often in potato while *D. angustula* was associated more with sweet corn and acorn squash.

The dominance distributions of Carabidae (Fig. 5) and Staphylinidae (Fig. 6) were highly skewed. In an agricultural setting, many epigeal taxa such as carabids (Tonhasca 1993) and staphylinids (Byers et al. 2000) often exhibit a dominance structure in which only a few species comprise a large proportion of the total abundance. This disparity seems to increase with agricultural intensification and loss of natural habitat (Desender et al. 1994; Kromp 1999). Tonhasca (1993) likened these assemblage characteristics to those of early successional communities because of the frequent disturbance of the agricultural habitat. This may be especially evident in small, diversified vegetable farms due to high disturbance levels associated with annual crop rotations and a variety of intensive pest management techniques.

The skewed dominance structure was readily apparent for Carabidae, where one species, *P. melanarius*, accounted for 73.5% of all the specimens. *P. melanarius* is an introduced generalist predator which has thrived in disturbed environments and has the

ability to out compete native species (Laroche and Larivière 2003). Temporal niche partitioning may explain the success of the other dominant carabid species. *P. melanarius* is an autumn breeding species, whereas many of the other abundant generalist predators in this study (*Bembidion quadrimaculatum oppositum* Say, *P. chalcites* and *P. lucublandus*) are spring breeding species. There is also evidence of temporal niche partitioning in another functional group: two well-known weed seed predators that were found in high abundance, *A. sanctaecrucis* (spring breeder) and *H. pensylvanicus* (autumn breeder), have similar trophic roles yet segregate temporally (Lundgren 2005).

The most abundant Staphylinidae (*D. angustula*, *H. lateralis*, and *S. ambigua*) all belong to the subfamily Aleocharinae, which, like many of the dominant carabid species, are considered generalist predators. The most dominant species shifted from *D. angustula* in 2001 to *H. lateralis* in 2002. This may indicate that *D. angustula* was better suited to the environment of the alfalfa crop in the years preceding the study resulting in a carryover effect in 2001, or possibly *H. lateralis* is better suited to deal with environmental disturbance and gradually surpassed *D. angustula* in dominance.

Biodiversity studies with invertebrates are often constrained due to the high number of species and corresponding difficulties with identification. Our work will help focus future epigeal coleopteran studies in agroecosystems by identifying, as a baseline, community members that are predominant in the Carabidae and Staphylinidae. We also provide a list of the seven formicid genera, including one species identification, collected during our study. While ants are primarily associated with tropical agroecosystems (Way and Khoo 1992), the large number of specimens found in diversified vegetable plots provides evidence that ants should be further studied in temperate agricultural systems to

better define their role. This study also shows that northeastern U.S. agroecosystems are not sterile communities, but contain a relatively high level of biodiversity.

This study indicates that implementing transgenic vegetable crops in diversified farm systems has little or no effect on species richness and dominance distribution of several dominant epigeal insect taxa. Additionally, genotype effects were not evident in multivariate analyses of community composition for Carabidae and Staphylinidae. Instead, crop type was a better predictor of variation in species assemblages. In diversified farm systems, longer-term, larger scale (e.g., approaching monoculture), or continuous-cropping studies may be needed to detect effects on biodiversity due to the deployment of transgenic crops. However, at least in the short term, the heterogeneity of crops coupled with crop rotation patterns common to diversified vegetable farms in northeastern US agroecosystems seems to aid in preserving epigeal biodiversity, which may be supplemented by reductions in insecticide use associated with transgenic crops.

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Table 1. Insecticide applications used on isoline and transgenic vegetable cultivars in diversified Pennsylvania farm plots during 2001 and 2002. Insecticide use was based on common preventative measures (at-planting applications) and pest threshold scouting.

Year	Genotype	Crop	Target Species	Product
2001	isoline	sweet corn	<i>Delia platura</i> (Meigen)	diazinon + lindane ^a
			<i>Helicoverpa zea</i> (Boddie)	lambda-cyhalothrin ^b
			<i>Helicoverpa zea</i> (Boddie)	lambda-cyhalothrin
		potato	<i>Leptinotarsa decemlineata</i> (Say)	imidacloprid ^c
			<i>Empoasca fabae</i> (Harris)	cyfluthrin ^d
		acorn squash	<i>Acalymma vittata</i> (Fabricius)	cyfluthrin
			<i>Anasa tristis</i> (DeGeer)	cyfluthrin
	transgenic	sweet corn	<i>D. platura</i>	diazinon + lindane
			<i>Popillia japonica</i> Newman	lambda-cyhalothrin
potato		<i>E. fabae</i>	esfenvalerate ^e	
		Aphididae	cyfluthrin oxamyl ^f	
acorn squash	<i>A. vittata</i>	cyfluthrin		
2002	isoline	sweet corn	<i>Helicoverpa zea</i> (Boddie)	lambda-cyhalothrin
			<i>Helicoverpa zea</i> (Boddie)	lambda-cyhalothrin
		potato	<i>Leptinotarsa decemlineata</i> (Say)	imidacloprid
			<i>Empoasca fabae</i> (Harris)	cyfluthrin
	transgenic	potato	<i>E. fabae</i>	cyfluthrin
		<i>E. fabae</i>	cyfluthrin	

^a Germate Plus, Trace Chemical LLC, Perkins, IL, 15% AI (diazinon), 25% AI (lindane), 2.23 g/kg seed

^b Warrior, Syngenta Crop Protection, Greensboro, NC, 11.4% AI, 222 ml/ha

^c Admire 2F, Bayer CropScience, Research Triangle Park, NC, 21.4% AI, 950 ml/ha

^d Baythroid, Bayer CropScience, Research Triangle Park, NC, 25% AI, 117 ml/ha

^e Asana XL, E. I. Dupont, Wilmington, DE, 585 ml/ha

^f Vydate L, E. I. Dupont, Wilmington, DE, 4677 ml/ha

Table 2. Coleoptera and Formicidae identified from Pennsylvania diversified vegetable farm plots in 2001 and 2002 (excluding Carabidae and Staphylinidae; see Tables 3 and 4.

Anthicidae	Cleridae	Lathrididae
<i>Anthicus cervinus</i> LaF.	<i>Enoclerus rosmarus</i> (Say)	<i>Melanophthalma cavicollis</i> Mannerheim
A. species 1 - 3		M. species 1 - 3
<i>A. thomasi</i> Pic.	Coccinellidae	Languriidae
<i>Notoxus anchora</i> Hentz	<i>Coccinella septempunctata</i> L.	<i>Toramus pulchellus</i> (LeConte)
<i>Tomoderus constrictus</i> (Say)	<i>Coleomegilla maculata</i> DeGeer	
Bruchidae	<i>Harmonia axyridis</i> Pallas	Mycetophagidae
	<i>Hippodamia convergens</i> Guerin	<i>Litargus balteatus</i> LeConte
Byrrhidae	<i>H. parenthesis</i> (Say)	<i>Typhaea stercorea</i> L.
<i>Curimopsis</i> species 1	<i>Psyllobora vigintimaculata</i> (Say)	
<i>C. strigosa</i> (Melsh.)	<i>Scymnus americanus</i> Mulsant	Nitidulidae
Byturidae	Corylophidae	<i>Carpophilus humeralis</i> (F.)
<i>Byturellus griseescens</i> (Jayne)	<i>Molamba lunata</i> (LeConte)	<i>C. lugubris</i> Murray
	<i>Sericoderus obscurus</i> LeConte	<i>C. sayi</i> Parsons
Cantharidae	S. species 1	<i>Glischrochilus fasciatus</i> (Olivier)
<i>Cantharis scitulus</i> Say	Cryptophagidae	<i>G. quadrisignatus</i> (Say)
Cerambycidae	<i>Anchicera ephippiata</i> Zimmermann	<i>Stelidota geminata</i> (Say)
<i>Elaphidion incertum</i> Newman	A. species 1	Phalacridae
Chrysomelidae	<i>Atomaria pumilio</i> Casey	<i>Acylopus ergoti</i> Casey
<i>Acalymma vittata</i> (F.)	A. species 1	<i>A. piceous</i> Casey
<i>Aphthona nigriscutis</i> Foundras	Species 1 - 4	<i>Phalacrus</i> species 1
<i>Chaetocnema confinis</i> Crotch	Cucujidae	<i>Stilbus apicalis</i> (Melsh.)
<i>C. cribrifrons</i> LeConte	<i>Telephanus velox</i> Haldemann	S. species 1
<i>C. denticulata</i> (Illiger)	Curculionidae	Ptiliidae
<i>C. minuta</i> (Melsh.)	Elateridae	<i>Ptilinus</i> species 1
<i>C. pulicaria</i> Melsh.	<i>Aeolus dorsalis</i> (Say)	Ptilodactylidae
<i>Diabrotica barberi</i> Smith & Lawr.	<i>Conoderus lividus</i> (DeGeer)	<i>Anchytarsus bicolor</i> (Melsh.)
<i>D. undecimpunctata howardi</i> Barber	Formicidae	<i>Ptilodactyla</i> species 1
<i>D. virgifera</i> Le Conte	<i>Camponotus</i>	Scarabaeidae
<i>Disonycha xanthomelas</i> (Dalmar)	<i>Formica</i>	<i>Ataenius spretulus</i> (Haldeman)
<i>Epitrix cucumeris</i> (Harris)	<i>Lasius</i>	<i>Onthophagus</i> species 1
<i>E. fuscata</i> Crotch	<i>Myrmica</i>	<i>Phyllophaga fraterna</i> Harris
<i>E. hirtipennis</i> (Melsh.)	<i>Prenolepis imparis</i> (Say)	<i>Popillia japonica</i> Newman
<i>E. humeralis</i> Drury	<i>Solenopsis</i>	<i>Serica sericea</i> (Illiger)
<i>E. species 1 - 2</i>	<i>Tetramorium</i>	Scolytidae
<i>Leptinotarsa decemlineata</i> (Say)	Histeridae	Silphidae
<i>Psylliodes punctulata</i> Melsh.	<i>Phelister subrotundus</i> Say	<i>Silpha americana</i> L.
<i>P. species 1</i>	<i>Saprinus assimilis</i> (Paykull)	Throscidae
<i>Phyllotreta ramosa</i> (Crotch)	Hydrophilidae	<i>Aulonothroscus convergens</i> Horn
<i>Systema frontalis</i> (Forster)	<i>Cercyon connivens</i> Fall	
Cicindellidae	Lampyridae	
<i>Cicindela punctulata</i> Olivier	<i>Photinus pyralis</i> (L.)	
Clambidae		
<i>Clambus</i> species 1		

Table 3. Carabidae species identified from Pennsylvania diversified vegetable farm plots in 2001 and 2002.

<i>Agonum cupripenne</i> (Say)	<i>Elaphropus anceps</i> (LeConte)
<i>A. muelleri</i> (Herbst)	<i>Harpalus affinis</i> (Shrank)
<i>A. placidum</i> (Say)	<i>H. caliginosus</i> Fabricius
<i>A. punctiforme</i> (Say)	<i>H. compar</i> LeConte
<i>Amara aenea</i> DeGeer	<i>H. erythropus</i> DeJean
<i>A. familiaris</i> (Duftschmid)	<i>H. faunus</i> Say
<i>A. impuncticollis</i> (Say)	<i>H. herbivagus</i> Say
<i>A. littoralis</i> Mannerheim	<i>H. longicollis</i> LeConte
<i>A. species 1</i>	<i>H. pensylvanicus</i> (DeGeer)
<i>Anisodactylus sanctaecrucis</i> Fabricius	<i>H. rubripes</i> (Duftschmid)
<i>Bembidion affine</i> Say	<i>Lebia viridis</i> Say
<i>B. impotens</i> Casey	<i>Microlestes linearis</i> (LeConte)
<i>B. obtusum</i> Audinet-Serville	<i>Notiobia terminata</i> (Say)
<i>B. patrulele</i> Dejean	<i>Notiophilus ceneus</i> (Herbst)
<i>B. quadrimaculatum oppositum</i> Say	<i>Patrobus longicornis</i> (Say)
<i>B. rapidum</i> (LeConte)	<i>Poecilus chalcites</i> (Say)
<i>B. versicolor</i> (LeConte)	<i>P. lucublandus</i> (Say)
<i>Bradycellus rupestris</i> (Say)	<i>Pterostichus melanarius</i> (Illiger)
<i>Calathus gregarius</i> (Say)	<i>Scarites quadriceps</i> Chaudoir
<i>Chlaenius tricolor tricolor</i> Dejean	<i>Stenolophus comma</i> (Fabricius)
<i>Clivina impressifrons</i> LeConte	<i>S. ochropezus</i> (Say)
<i>Cymindis limbata</i> Dejean	<i>S. species 1</i>
<i>Dicaelus elongatus</i> Bonelli	<i>Trechus quadristriatus</i> (Schrank)
<i>Dyschirius globulosus</i> Say	

Table 4. Staphylinidae species identified from Pennsylvania diversified vegetable farm plots in 2001 and 2002.

<i>Acylophorus</i> species 1	<i>Neohypnus</i> spp.
<i>Aleochara curtula</i> (Gavenhorst)	<i>Paederus littorarius</i> Gravenhorst
<i>A. gracilicornis</i> Bernhauer	<i>Philonthus concinnus</i> (Gravenhorst)
<i>A. verna</i> Say	<i>P. debilis</i> (Gravenhorst)
<i>Anotylus</i> species 1	<i>P. species</i> 1
<i>A. tetracarinatus</i> Block	<i>Platystethus</i> species 1
<i>Astenus longiusculus</i> Mannerheim	<i>Scaphisoma convexum</i> Say
<i>Belonuchus rufipennis</i> (Fabricius)	<i>S. suturale</i> LeConte
<i>Dinaraea angustula</i> (Gyllenhal)	<i>Stenistoderus rubripennis</i> (LeConte)
<i>Falagria dissecta</i> Erichson	<i>Stenus</i> species 1
<i>Gabrius nigrutilus</i> (Gravenhorst)	<i>Strigota ambigua</i> (Erichson)
<i>Hoplandria lateralis</i> (Melsheimer)	<i>Tachinus fimbriatus</i> Gravenhorst
<i>Lobrathium collare</i> Erichson	<i>Tachyporus nitidulus</i> (Fabricius)
<i>Neobisnius sobrinus</i> (Erichson)	<i>Tinotus</i> species 1
<i>Neohypnus obscurus complex</i> (Erichson)	

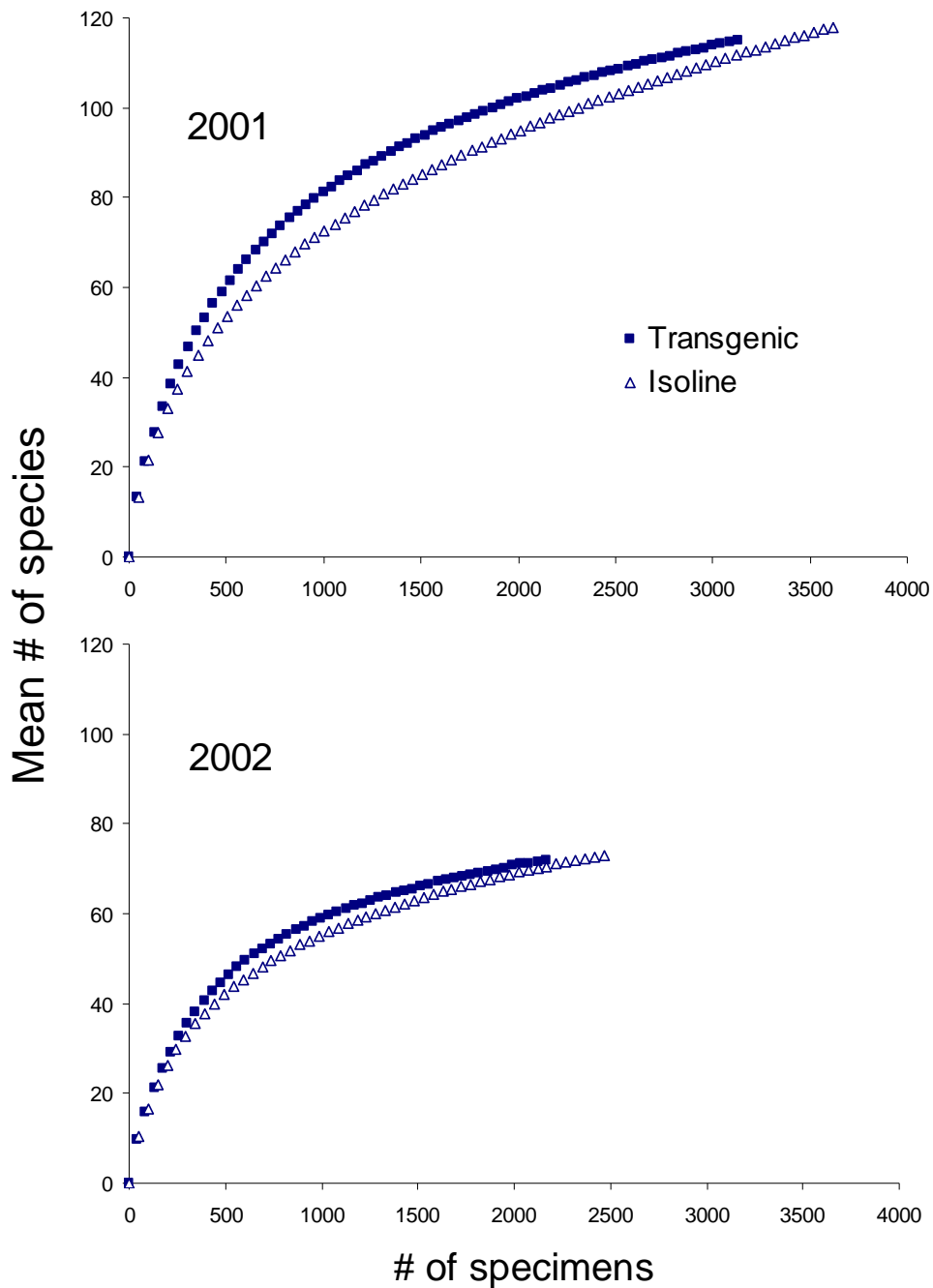


Figure 1. Species rarefaction curves for epigeal beetles and ants found in transgenic and isoline diversified vegetable farm systems consisting of sweet corn, potatoes, and acorn squash in 2001, and sweet corn and potatoes in 2002 (based on 50 permutations of data). Expected number of species is plotted against number of individuals. 95% confidence intervals (not shown) are overlapping for all transgenic vs. isoline comparisons.

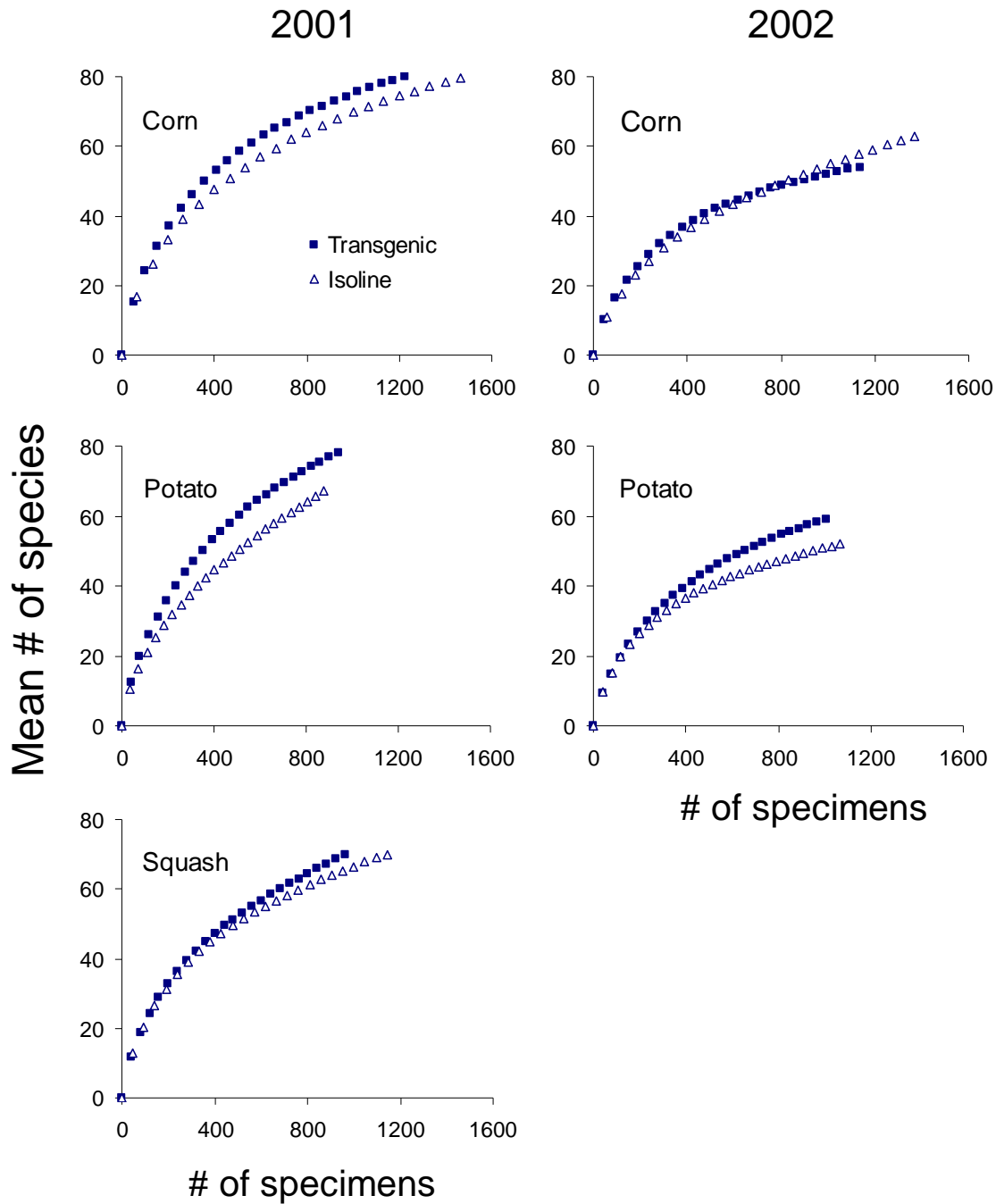


Figure 2. Species rarefaction curves for epigeal beetles and ants found in transgenic and isoline sweet corn, potato and acorn squash in 2001, and sweet corn and potatoes in 2002 (based on 50 permutations of data). Expected number of species is plotted against number of individuals. 95% confidence intervals (not shown) are overlapping for all transgenic vs. isoline comparisons.

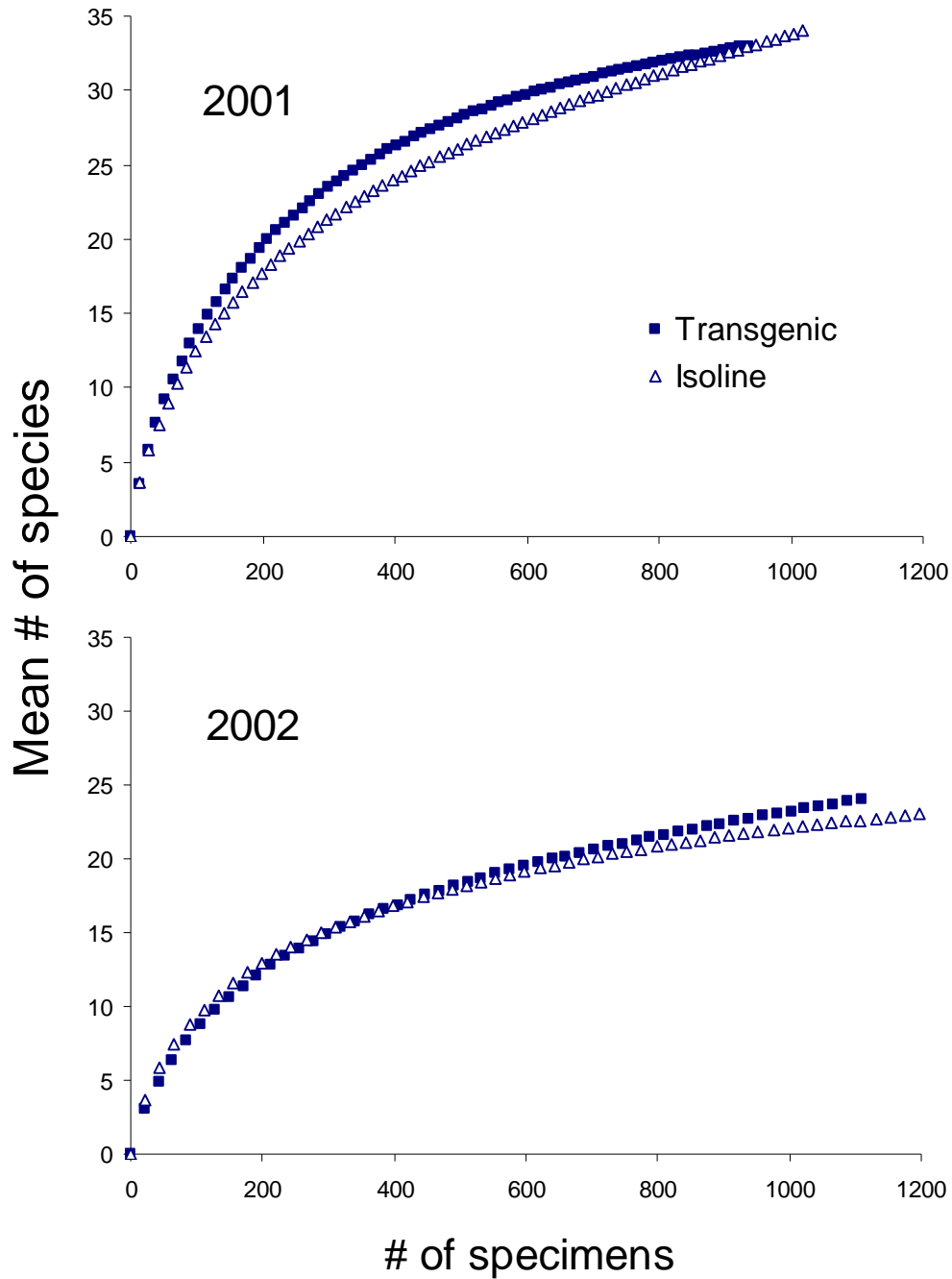


Figure 3. Species rarefaction curves for Carabidae found in transgenic and isoline diversified vegetable farm systems consisting of sweet corn, potatoes, and acorn squash in 2001, and sweet corn and potatoes in 2002 (based on 50 permutations of data). Expected number of species is plotted against number of individuals. 95% confidence intervals (not shown) are overlapping for all transgenic vs. isoline comparisons.

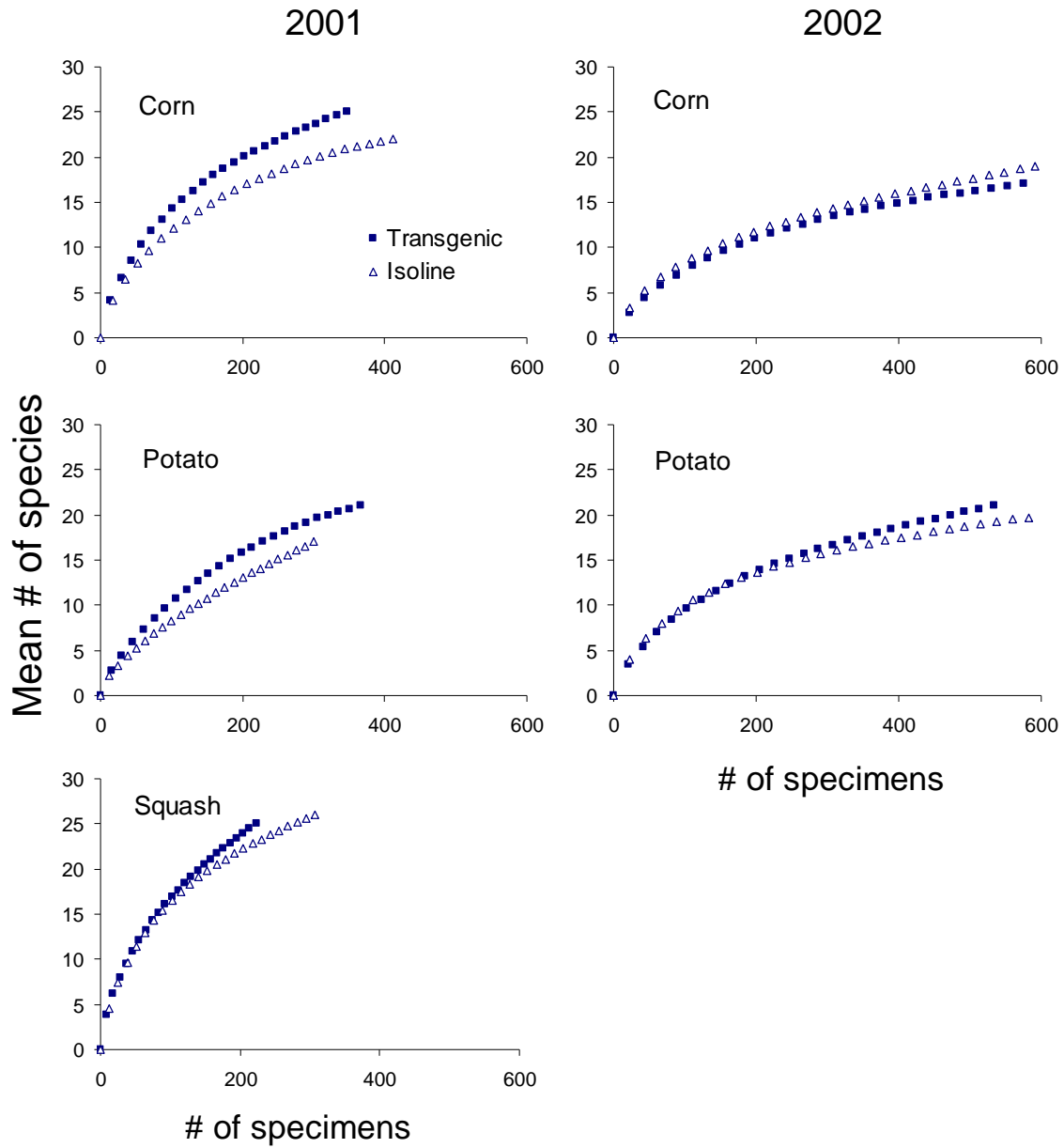


Figure 4. Species rarefaction curves for Carabidae found in transgenic and isoline sweet corn, potato and acorn squash in 2001, and sweet corn and potato in 2002 (based on 50 permutations of data). Expected number of species is plotted against number of individuals. 95% confidence intervals (not shown) are overlapping for all transgenic vs. isoline comparisons.

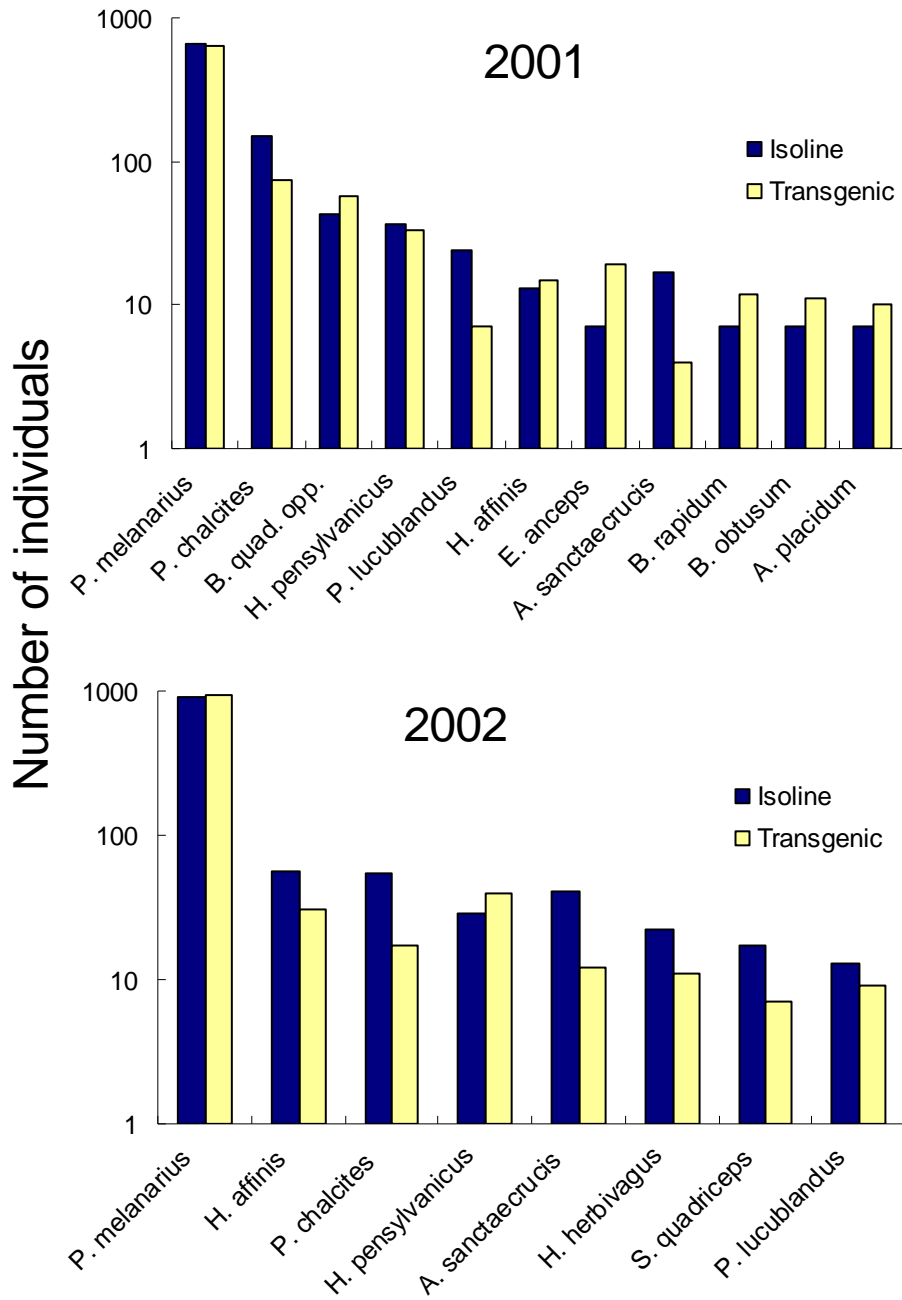


Figure 5. Rank abundance (on a log₁₀ scale) of Carabidae in transgenic and isoline diversified farm systems in 2001 and 2002. Species shown represent at least 1% of total abundance.

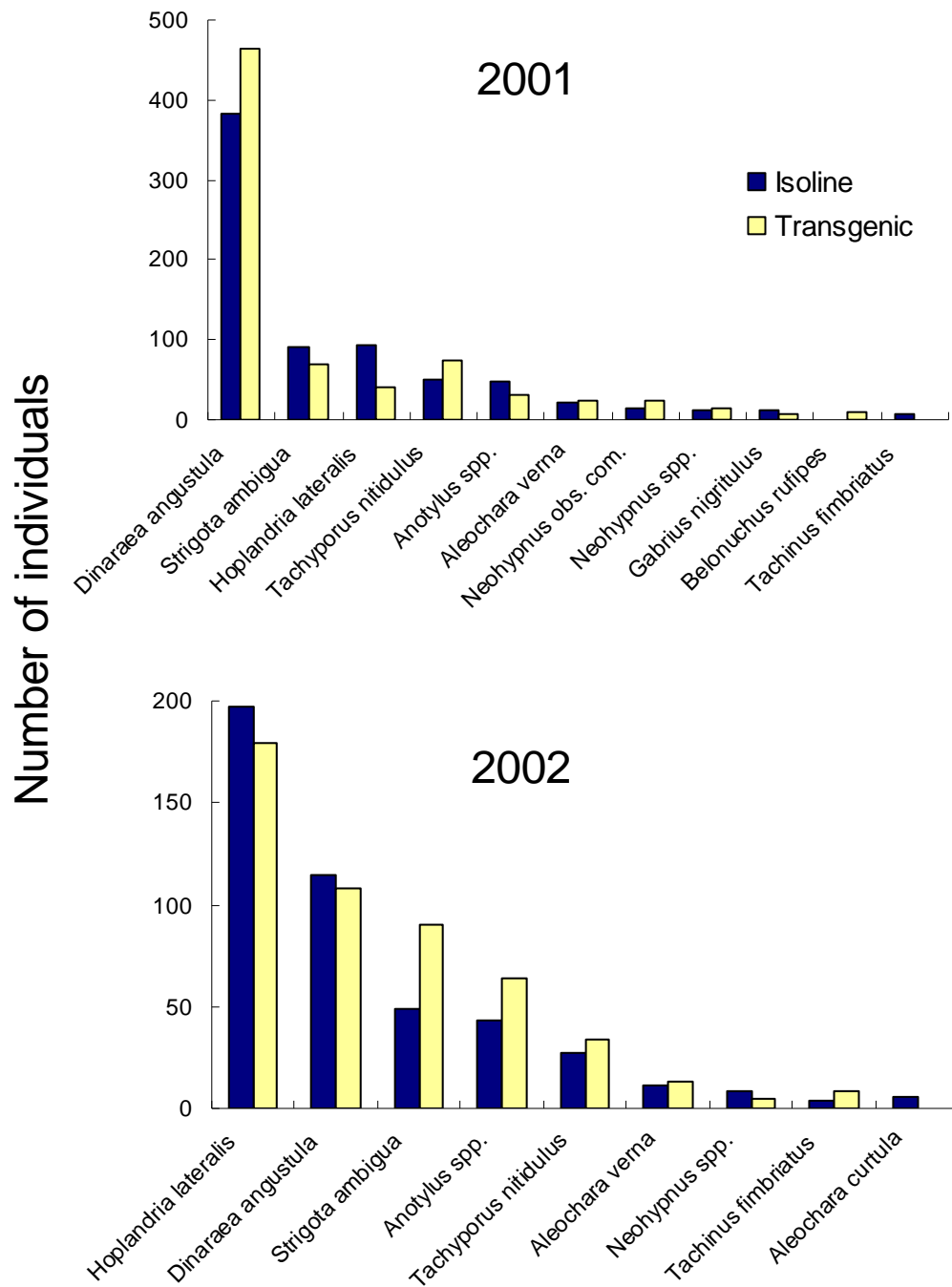


Figure 6. Rank abundance of Staphylinidae in transgenic and isoline diversified farm systems in 2001 and 2002. Species shown represent at least 1% of total abundance.

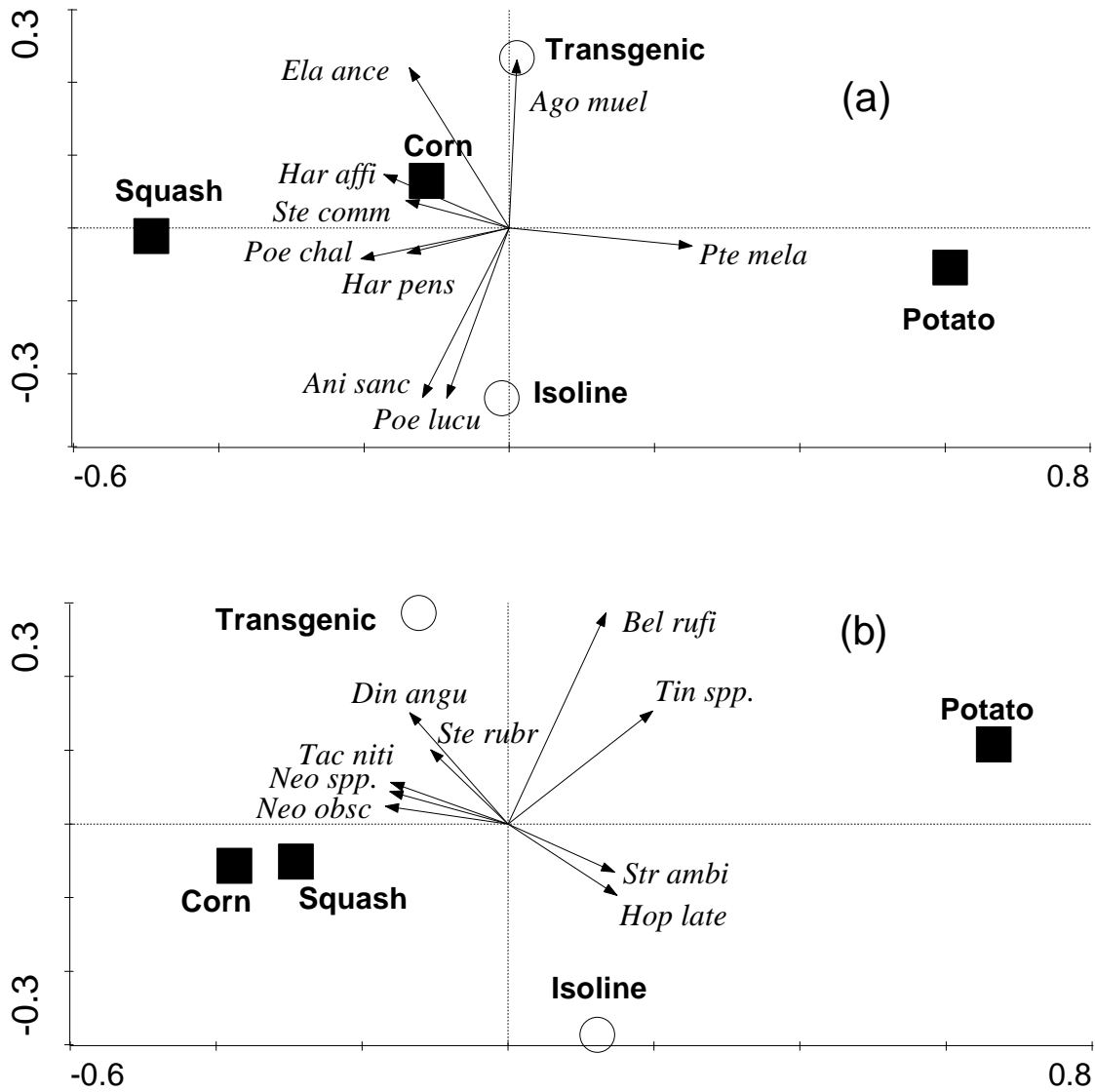


Figure 7. Redundancy analysis of the most abundant (a) Carabidae and (b) Staphylinidae in relation to nominal treatment variables in 2001. For both families, potato ($P = 0.002$) had a significant influence on community composition and is represented by the first (x-) axis (potato versus corn and squash). The second (y-) axis is represented by genotype (isoline versus transgenic). The nine most influential species are shown in each bi-plot. Species, shown as arrows, are denoted by the first three letters of the genus and the first four letters of the species (see Table 3 and Table 4 for full names).

Chapter 3

Carabidae population dynamics and temporal partitioning: response to coupled neonicotinoid-transgenic technologies

ABSTRACT

The adoption of insecticidal *Bt* crops has been accompanied with ecological studies examining effects to non-target organisms. During a two-year study, we examined the dominance structure of carabids in maize (both sweet and field corn) agroecosystems and compared the population dynamics of the most abundant species between management systems using transgenic technology, conventionally managed maize, and a control (i.e. no insect control methods). Transgenic sweet corn contained Cry1Ab/c proteins, while transgenic field corn contained the coupled technology of Cry3Bb1 proteins for control of corn rootworm and neonicotinoid seed treatments aimed at secondary soil-borne pests. The carabid community was dominated by four species, *Scarites quadriceps* Chaudoir, *Poecilus chalcites* Say, *Pterostichus melanarius* Illiger, and *Harpalus pensylvanicus* DeGeer, that each occupied a distinct temporal niche during the growing season. Two species, *P. melanarius* and *H. pensylvanicus*, exhibited differences between treatments over time. Only *H. pensylvanicus* had consistent results in both years, in which activity-densities in field corn were significantly higher in the control than in transgenic or conventionally managed treatments in July and/or August. These results, along with laboratory bioassays (Mullin et al. 2006), led us to hypothesize that lower adult captures resulted from exposure of *H. pensylvanicus* larvae to soil-directed insecticides - either the neonicotinoid seed treatment in the transgenic field corn, or an at-planting soil insecticide in the conventional field corn.

INTRODUCTION

The use of genetically engineered (GE) crops has risen steadily over the past decade and adoption has been highest in U.S. agriculture, accounting for approximately 59% of the 200 million acres planted worldwide in 2004 (USDA 2006). While most of the GE crop acreage consists of herbicide-tolerant crops, insect-resistant (*Bt*) varieties of cotton and maize have established a firm foothold in U.S. agriculture. In 2006, *Bt* maize represented 40% of total U.S. maize acreage and consisted of cultivars expressing Cry1- or Cry3-based endotoxins used to control European corn borer and corn rootworm, respectively (NASS 2006). The introduction of *Bt* crops has been accompanied with concern for negative effects to non-target insects (Hails 2000, Groot and Dicke 2002), especially those in the same insect order for which the Cry proteins are directed or for important biological control agents found in agricultural fields. Numerous studies in both the field and the laboratory have examined such effects for a variety of invertebrates, including (among others): butterflies (Losey 1998, Wraight et al. 2000), lacewings (Hilbeck et al. 1998), ladybird beetles (Al-Deeb and Wilde 2003, Bai et al. 2006), and ground-dwelling beetles (Duan et al. 2004, Mullin et al. 2006, Leslie et al 2007).

Comparing population dynamics of non-target organisms between farm systems utilizing transgenic versus conventional pest management practices may elucidate underlying interactions between the transgenic technology and the ecology of the surrounding habitat. Groot and Dicke (2002) noted that non-target species could come in contact with *Bt* toxins by feeding directly on *Bt* plants, feeding on target or non-target herbivorous insects, or through the environment (e.g., accumulation of toxins in the soil). Introduced transgenes can also alter pest management inputs, thereby indirectly affecting

non-target organisms and farmland biodiversity (Hails 2000, Leslie et al. 2007). For example, lepidopteran-specific (Cry1-based) transgenic maize and cotton can eliminate or reduce pyrethroid applications directed at European corn borer and pink bollworm, respectively. Similarly, coleopteran-specific (Cry3-based) transgenic maize may shift insecticide use patterns from at-planting applications of soil insecticides toward seed treatments. The efficient control of target pests by *Bt* crops can alter trophic relationships as well: Riddick et al. (1998) found lower abundances of the carabid, *Lebia grandis* Hentz, a specialist predator of *Leptinotarsa decemlineata* (Say), in transgenic potato fields designed to control *L. decemlineata*, while the abundance of the generalist predator, *Coleomegilla maculata* DeGeer, was not affected due to its ability to feed on alternate prey.

Changes in pest management tactics are rarely introduced independently or in isolation. For example, all commercially available Cry3-based field corn contains neonicotinoid seed treatments for control of secondary soil-borne pests (Smith et al. 2004). While the majority of studies have found little or no direct non-target effects after exposure to transgenics (summarized by Romeis et al. 2006), other technology associated with transgenics, such as seed treatments, could affect non-target organisms. Recently, Mullin et al. (2006) identified neonicotinoid seed treatments found on transgenic maize as a major mortality factor for carabids in laboratory bioassays, and alternatively could find no negative effects for Cry1Ab/c or Cry3Bb1 toxins delivered to carabids in transgenic corn pollen. We therefore introduce the term “transgenic technology” for the transgenic treatments in our study to depict the realistic evaluation of coupled technologies such as transgenes and neonicotinoid seed treatments.

Ground beetles, or Carabidae, have been used as bioindicators for agricultural practices due their sensitivity to changes in the environment, abundance in agricultural settings, diversity of functional roles and ease of sampling using pitfall traps (Duelli 1999). Many carabids are voracious feeders and are often grouped as invertebrate predators or herbivores (e.g., weed seed predators), although supplementary feeding such as scavenging dead insects qualifies most carabids as omnivorous (Toft and Bilde, 2002). Therefore it is highly likely that carabids will directly or indirectly come into contact with *Bt* toxins in *Bt* crop fields. Indeed, Zwahlen and Andow (2005) were able to detect Cry1Ab in carabids collected from fields with *Bt* crops or crop residues. However, ingestion of *Bt* toxins, even Cry3-based coleopteran-specific toxins, seems to be of little direct threat to carabids (Mullin et al. 2006). Neonicotinoid seed treatments (Mullin et al. 2006), conventional insecticide applications (Carcamo et al. 1995), or indirect effects may be of more concern to carabid communities in the field.

Northeastern U.S. agroecosystems, where this study was conducted, are characterized by a complex mix of numerous small farms nested in heterogenous landscapes, of which maize is often a component of dairy and vegetable production. Therefore, maize for animal feed and sweet corn are prominent features of these landscapes, and are often in close proximity, resulting in mosaics of varying types of maize. Pest management inputs vary dramatically among these types of maize. At-planting soil insecticides, primarily directed for control of corn rootworm, dominate inputs in maize grown for animal feed. Foliar insecticide use later in the season is highest in sweet corn, where several ear-damaging insects can severely limit production as described in Crop Profiles and Pest Management Strategic Plans of states throughout

the sweet corn growing region of the U.S. (see, www.northeasternipm.org). Adding complexity to the system, these maize pest management inputs can be replaced or altered by the use of transgenic-technologies.

The differential effects to Carabidae for each pest management regime would not be easily detectable using only family-level identification or a single species (Lopez et al 2005). Instead, identifying when and where abundant carabid species are occurring and choosing from these a range of species encapsulating the whole growing season would be the most useful approach for examining non-target effects (Wiles and Barrett 1998). Carabids are abundant in agricultural settings throughout the entire temperate growing season and the dominant species vary temporally due to life history characteristics (Holland 2002).

Here, we characterized the carabid community in Pennsylvania maize fields over two years and identified feasible candidates as bioindicators for pest management effects in maize. Candidates were chosen based on their level of activity-density (high numbers needed for statistical analysis purposes) and their seasonality, to cover the range of times that pest management events occur. Carabids were sampled weekly to look for finer temporal partitioning than “spring” and “autumn” breeders and to identify species active during discrete pest management events. We examined the population dynamics of the candidate species to compare differences between transgenic technologies, additional soil or foliar insecticide inputs (insecticides vs. no insecticides) and types of maize (sweet corn vs. field corn).

METHODS

Experimental design. A replicated, split plot experiment was employed to examine the effects of transgenic technology (transgenic technology vs non-transgenic) and insecticide input (insecticides vs no insecticides) on Carabidae in sweet corn and field corn. The experiment was conducted on the Penn State research farms at the Russell E. Larson Agricultural Experimental Station in Pennsylvania Furnace, PA and spanned the 2003 and 2004 growing seasons. The study site contained Hagerstown silt loam soils with 0 to 3 percent slopes (HaA). In each year, we used twelve 1-acre (0.4 ha) experimental units, representing four treatments replicated three times. All experimental units were surrounded by a 3 m alfalfa border. Each experimental unit was comprised of randomly assigned field corn, sweet corn and snap bean subplots (each 55m x 18.3m). Snap beans were present as a rotational crop for sweet corn in the second year, whereas field corn was not rotated as is common practice in this growing region. The four treatments (Table 1) were defined by transgenic technology and insecticide management, and were described as: 1) Maize utilizing transgenic technology (TT), 2) maize utilizing transgenic technology with conventional insecticides (TT + Ins), 3) isoline maize with conventional insecticides (Iso + Ins), and 4) isoline maize without insecticides (Iso), i.e., control.

In the insecticide management treatments, field corn and sweet corn received an at-planting soil application of the pyrethroid, tefluthrin (Force 3G, Syngenta, Greensboro, NC, 3% AI, 3.7 kg/ha), for control of corn rootworm and other seed- and root- feeding insects. Additionally, insecticide managed sweet corn received four late-season foliar applications of the pyrethroid, lambda-cyhalothrin (Warrior, Syngenta, Greensboro, NC,

11.4% AI, 222 ml/ha), for control of lepidopteran ear-feeding pests. These pre-determined insecticide-use patterns were consistent with northeastern maize pest management.

Our choice of transgenes focused on those that were commercially available and most likely to influence insecticide use patterns in the U.S. For field corn, we used transgenic cultivars expressing Cry3Bb toxins, aimed at root-feeding stages of *Diabrotica* spp. These Cry3 cultivars are expected to reduce broad-spectrum soil insecticides, but as mentioned above, these are coupled with neonicotinoid seed treatments to control non-rootworm damage to seeds, seedlings, and roots. Specifically, for the field corn transgenic-technology treatment, DeKalb DKC60-12 YGRW (Monsanto Co., St. Louis, MO) field corn expressed Cry3Bb1 endotoxins for control of corn rootworm and also contained a neonicotinoid seed treatment [160 µg imidacloprid / seed, or 250 µg clothianidin / seed (Bayer CropScience, Research Triangle Park, NC)] for control of secondary seed- and root-feeding pests. All commercially available *Bt* field corn varieties contains neonicotinoid seed treatments, thus this evaluation assesses the effect of this packaged “transgenic technology” on Carabidae dynamics. In the field corn experimental units that did not use transgenic-technology, we planted the near isolate, DKC60-17, which does not contain a neonicotinoid seed treatment. For sweet corn we used cultivars expressing Cry1Ab/c toxins, aimed primarily at European corn borer, which is expected to reduce broad-spectrum foliar insecticides. Rogers (Syngenta Seeds, Boise, ID) Attribute WSS 0984 sweet corn expressing Cry1Ab/c endotoxins for control of European corn borer was used for the transgenic treatment, while Boreal F1 sweet corn was the near isolate. The aforementioned insecticide use patterns and transgenic

cultivars were also used in the combined treatment (transgenic technology + insecticide management). Additionally, all corn seed used was treated with a fungicide blend shown previously not to be directly toxic to Carabidae in the laboratory (Mullin et al. 2005).

Insect collection and identification. Pitfall traps were used to capture all epigeal coleopteran specimens. The pitfall traps were made from plastic deli containers, 14.1 cm deep with 10.9 cm inside diameter, and were inserted flush with the ground. Another small collection cup (5.5 cm deep, 8.2 cm inside diameter) filled with ethylene glycol was placed inside for removal of specimens. An inverted top of a 2-liter soda bottle was put in each deli-container to act as a funnel, limiting escape by beetles and access of the ethylene glycol to small mammals. Ten traps, arranged in two transects of five, were placed at 9, 18, 27, 36 and 45 m within the rows of all field corn and sweet corn subplots for a total of 240 traps. Each subplot of field corn and sweet corn had twenty-two rows and the traps were placed in row numbers eight and fourteen from the south side of the subplot. In 2003, trapping consisted of 11 sampling dates between June 12 – Sept 7. In 2004, there were 12 trapping dates extending from May 27 – Sept 15. Trapping occurred every 7-14 days depending on weather conditions. For each sampling period, one row of five traps was opened in each field corn and sweet corn subplot for a total of 120 open traps. The open rows were alternated each week to avoid over-sampling a portion of the field. All traps were open for 72 hours, after which all beetles were transferred to 70% ethanol. Beetles were later sorted, pinned and identified to species. Identifications were performed by Robert Davidson (Carnegie Museum of Natural History, Pittsburgh, PA), David Biddinger (Pennsylvania State University) and Tim Leslie (Pennsylvania State University), using several taxonomic keys (Downie and Arnett Jr. 1996, Ciegler 2000,

Marshall 2006) and voucher specimens from previous studies (Hoheisel 2002, Leslie et al. 2007). Voucher specimens are housed in the lab of S. J. Fleischer, Department of Entomology, Pennsylvania State University.

Data analysis. Data were recorded as average activity-density (# of beetles / trap / 72 hours) for each species at the subplot-level. A complete species list was compiled and species were plotted in order of dominance. Species with the highest activity-density (> 200 individuals in each of the two years) were chosen to examine year-to-year temporal patterns and treatment effects on population dynamics. All analyses of treatment effects were performed separately for each crop and year, to avoid confounding effects of crop rotation and pest management inputs between sweet corn and field corn. Therefore, a repeated-measures proc mixed procedure (SAS v.9.1.3) was conducted on each species to examine the effect of date, transgenic technology, insecticide use, and their interactions. Replicate was included in the analysis as a random variable. Post hoc Tukey means separation tests were used to identify the direction of the treatment effects on activity-densities.

RESULTS

Over the two-year study, 7,256 Carabidae representing 49 species (Table 2) were collected, a higher number than is generally recorded (~ 30 species) for temperate cropping regions (Luff 2002). Dominance distribution was strongly skewed with 10 species accounting for > 75% of the total abundance (Fig. 1). *Scarites quadriceps* Chaudoir and *Pterostichus melanarius* Illiger were the most abundant species in 2003 and

Harpalus pensylvanicus DeGeer, *Poecilus chalcites* Say, and *Bembidion*

quadrinotatum oppositum Say were the most abundant species in 2004 (Fig. 1).

Of the most abundant species, *H. pensylvanicus*, *S. quadriceps*, *P. chalcites* and *P. melanarius* were the only species with > 200 individuals collected in each of the two years. Date was significant (repeated measures ANOVA, $\alpha = 0.05$) for all four species in both years as depicted by shaded areas in Figure 2. The four species were most abundant during different times of the season; *S. quadriceps* emerged first, followed by *P.*

chalcites, *P. melanarius*, and *H. pensylvanicus* (Fig. 2). This pattern held true in both years except for *H. pensylvanicus* emerging earlier in 2004 and remaining abundant for the remainder of the season (Fig. 2). Table 3 shows significant treatment effects for the most abundant carabids in each crop and year, which are summarized below.

Scarites quadriceps. *S. quadriceps*, a spring breeder, was consistently the first species to emerge with activity-densities highest in June and early July and very few individuals trapped after mid-July (Figure 2). *S. quadriceps* did not respond to insecticide treatments across dates, although mean activity-density was higher in *Bt* sweet corn than isoline sweet corn in 2003 ($F_{1,78} = 4.15$, $P = 0.045$).

Poecilus chalcites. *P. chalcites*, another spring breeder, reached peak activity-density after *S. quadriceps* with highest records from late June through the end of July (Fig. 2). No significant treatment effects were detected in 2003, however, in 2004 *P. chalcites* mean activity density differed between treatments in both field and sweet corn. In 2004, mean activity-densities were significantly higher in field corn receiving no insecticide inputs, or the isoline unmanaged plots ($F_{1,86} = 9.36$, $P = 0.003$). *P. chalcites* mean activity-density also varied between insecticide management regimes in sweet corn in

2004 ($F_{1,86} = 4.95$, $P = 0.0287$) with highest mean activity-density recorded in the *Bt* managed sweet corn.

***Pterostichus melanarius*.** *P. melanarius*, an autumn breeder, peaked in activity density during August (Fig. 2). Treatment effects were only detected in 2003. *P. melanarius* exhibited a significant date by treatment interaction in sweet corn ($F_{9,78} = 2.92$, $P = 0.0049$), with significantly higher activity-densities found in the isoline unmanaged treatment for three dates in August and September (Figure 3, indicated with asterisks) based on Tukey mean comparisons ($\alpha = 0.05$). No date by treatment interactions were significant in field corn, however, mean activity-density varied between treatments ($F_{1,78} = 6.89$, $P = 0.0104$) with highest activity-densities in *Bt* managed and isoline unmanaged field corn.

***Harpalus pensylvanicus*.** The autumn-breeding *H. pensylvanicus* was the most dominant species (Fig. 1), exhibited the highest activity density in August and September (Fig. 2), and was rarely found before mid-July. *H. pensylvanicus* responded to insecticide management inputs in field corn in both 2003 ($F_{9,78} = 2.54$, $P = 0.0129$) and 2004 ($F_{10,86} = 2.01$, $P = 0.0419$). *H. pensylvanicus* activity-densities were significantly higher in the isoline unmanaged treatment for dates in September of 2003 and in July and September of 2004 (Figure 4, indicated with asterisks) based on Tukey mean comparisons ($\alpha = 0.05$).

DISCUSSION

Little evidence to date exists that suggests transgenes conferring the expression of *Bt* Cry toxins directly affects non-target organisms (Romeis et al. 2006). Instead, it is more

likely that dynamic shifts in insecticide use patterns and delivery systems due, in part, to the deployment of transgenic crops may influence non-target populations and communities through direct or indirect effects (Marvier et al. 2007). This study identified prominent carabid species in Pennsylvania maize agroecosystems and provided field evaluations of transgenes, some of which are coupled with neonicotinoid seed treatments, and conventional pest management practices (i.e. soil and foliar insecticide applications) on these populations. Working in conjunction with laboratory bioassay studies (i.e., Mullin et al. 2006), which identified neonicotinoid seed treatments present in Cry3-protected field corn, but not the Cry3 protein directly, as a significant direct mortality factor for carabids, we employed a range of insecticide management options relevant to current conditions.

Over the two-year study, four species (i.e., *S. quadriceps*, *P. chalcites*, *P. melanarius*, and *H. pensylvanicus*) exhibited high activity densities in each of the two years (>200 total individuals or 0.15 individuals / trap / 72 hr) and dominance distribution was highly skewed as the ten most abundant carabid species (out of the 49 total species) accounted for greater than 75% of all specimens captured. These results can be compared with other studies of carabids in different Pennsylvania agricultural systems. In a diversified vegetable farm setting, Leslie et al. (2007) found comparable species richness (i.e., 47 species) and similar abundant species, except for far fewer *S. quadriceps*. It is likely that fewer *S. quadriceps* were found in this earlier study at a similar location due to the fact that pitfall trapping began shortly after the period of peak activity-density for this species, highlighting the importance of temporal trapping considerations when studying carabid communities. A study examining carabid

communities in Pennsylvania grazed dairy pastures also reported similar species richness (i.e., 44 species) but differed in community composition (Byers et al. 2000). Whereas species such as *P. chalcites* and *P. melanarius* were abundant in both systems, *H. pensylvanicus* was found in much lower numbers in the grazed pasture, whereas *Amara aenea* DeGeer was the most dominant species in the grazed pasture and relatively scarce in the corn. Both *H. pensylvanicus* and *A. aenea* are weed seed predators and the local weed communities, which can vary greatly between agricultural systems (Davis et al. 2005), may have selected for or against each species based on their feeding preferences.

Activity-densities were noticeably higher in 2004 (3.27 beetles / trap / 72 hr) than in 2003 (2.14 beetles / trap / 72 hrs), primarily due to a three-fold increase in *H. pensylvanicus* activity-densities as well a three- to four-fold increase in some smaller bodied carabids such as *B. quadrimaculatum oppositum* and *B. rapidum*. With the exception of *H. pensylvanicus*, more smaller-bodied carabids were found among the most abundant beetles in the second year. Büchs et al (2003) suggest that smaller bodied insects may adapt better to disturbed environments and our findings seem to support this phenomenon. *H. pensylvanicus*, while larger-bodied, most likely increased in activity-density due to higher weed densities, primarily giant foxtail (*Setaria faberi*) and yellow foxtail (*Setaria glauca*), in the experimental fields during the second year (not measured, personal observation). Feeding preference trials have shown that foxtail is a preferred seed for *H. pensylvanicus* (Best and Beegle 1977, Lund and Turpin 1977)

Interestingly, the four species abundant in both years exhibited unique temporal niches based on the period of their highest activity-density. Carabids are generally grouped as spring breeders and autumn breeders with recognition of annual variation or

species-specific variation in life cycles (Thiele 1977, Loreau 1985, Holland 2002). For example, it would be known *a priori* that *S. quadriceps* and *P. chalcites* are spring breeders and *P. melanarius* and *H. pensylvanicus* are autumn breeders before our study was conducted. However, we have established that, not only are these four species found in high numbers in Pennsylvania agroecosystems (see also Leslie et al. 2007), but that they seem to occupy temporal niches at a finer resolution than the generalized seasonal depictions, resulting in a temporal succession. In general, this succession consisted of *S. quadriceps*, *P. chalcites*, *P. melanarius*, and *H. pensylvanicus* (Figure 2). In one exception, *P. melanarius* and *H. pensylvanicus* overlap in abundant periods in 2004, however these two species occupy different functional niches as well: *P. melanarius* is considered primarily carnivorous (Sunderland 2002), while *H. pensylvanicus* is a well-known weed seed predator (Tooley and Brust 2002). Therefore, the dominant carabid species seem to have evolved toward segregating resources both temporally and in feeding preferences. Detailed knowledge of temporal partitioning can be useful in choosing appropriate bioindicator species for examining external inputs that occur at distinct times in the field season (e.g. at-planting soil insecticide applications).

Treatment effects on the population dynamics of the most abundant carabids were only consistent for *H. pensylvanicus*. For several sampling periods in both years, *H. pensylvanicus* exhibited significantly higher activity-densities in isoline field corn that had no insecticide input (Figure 4). The other field corn treatments either contained an at-planting soil insecticide or the transgenic technology, which contained the neonicotinoid seed treatment. All insecticide management inputs occurred early in the season when the less-mobile *H. pensylvanicus* larvae would be feeding and in close

proximity to the corn seed in the soil. The possible insecticide-induced mortality factors seem to be evident in the trap captures of adults later in the season. *P. melanarius* was the only other species exhibiting a treatment effect on its population dynamics, with activity-densities higher in isoline sweet corn without insecticides for three sampling periods in 2003. As with *H. pensylvanicus*, early-season treatments may have affected *P. melanarius* larval abundance, or late season foliar sprays may have directly affected *P. melanarius* adults. This effect, however, was not evident in 2004. Sweet corn was rotated and probably caused homogenizing effects on ground beetle communities where treatment effects are lost due to population legacy at that position in the field (Leslie et al. 2007).

Treatment effects were evident when looking at the main effects of transgenic technology or insecticide use (or their interactions) on mean activity density (Table 2), but these results were not consistent and sometimes contradictory. For example, in 2004, *P. chalcites* exhibited a higher mean activity-density in isoline field corn unmanaged with insecticides, but in sweet corn had the highest mean activity-density in *Bt* sweet corn managed with insecticides. In another study examining Cry3-protected field corn under different insecticide use regimes, Bhatti et al. 2005 found lower densities of carabids in maize treated with seed-, soil- and foliar-applied insecticides (compared to no insecticide use) but analyses were performed at the family level and results were also not consistent across all years and trap types. Lopez et al. (2005) suggest that spatial and temporal variability combined with the limited power of most experimental designs can lead to such variability or lack of treatment effects in results. Additionally, insecticide inputs

can cause variation in behavior such as increased activity or dispersal (Wiles and Jepson 1994, Singh et al. 2001), which may affect trap captures.

Conclusions. Several carabid species exhibited high activity densities and occupied distinct temporal niches in Pennsylvania maize agroecosystems, making all possible bio-indicator candidates for studies addressing maize management in northeastern agroecosystems. Of these species, *H. pensylvanicus* exhibited the strongest response to maize insect pest management inputs in field corn. *H. pensylvanicus* activity-density was consistently higher in isoline field corn untreated with insecticides, whereas the lower population levels in the other treatments - conventional insecticide use, transgenic technology (Cry3Bb1 proteins + neonicotinoid seed treatment), or a combination of both – were indistinguishable from one another. Despite the coleopteran-specificity of Cry3-proteins in *Bt* field corn, all indications are that these toxins present no direct mortality threat to carabids (Al-Deeb and Wilde 2003, Mullin et al. 2006). However, the neonicotinoid seed treatments that accompany *Bt* field corn were determined a clear mortality factor in laboratory bioassays (Mullin et al. 2006) and the results of this study suggest that this mortality factor may be detectable in a field setting for at least one carabid species, *H. pensylvanicus*. Despite labor-intensive requirements, field studies that stress strong temporal and taxonomic resolution offer the best possibility for detection of non-target effects and could be further strengthened through meta-analyses (e.g., Marvier et al. 2007) that increase statistical power and may allow for examination of less common species.

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Table 1. Pest management inputs defining the experimental treatments for two types of maize.

Treatment	Type of maize	
	Field Corn	Sweet Corn
Control	None	None
Conventional insecticides	Pre-emergence soil insecticide ¹	Pre-emergence soil insecticide ¹ Foliar applications during silking growth stage ⁴
Transgenic technology	Cry3Bb1 toxins ² Neonicotinoid seed treatment ³	Cry1Ab/c toxins ⁵
Conventional insecticides and transgenic technology	Pre-emergence soil insecticide ¹ Cry3Bb1 toxins ² Neonicotinoid seed treatment ³	Pre-emergence soil insecticide ¹ Foliar applications during silking growth stage ⁴ Cry1Ab/c toxins ⁵

¹ Tefluthrin (Force 3G), Syngenta, Greensboro, NC, 3% AI, 3.7 kg/ha

² DeKalb DKC60-12 YGRW (Monsanto Co., St. Louis, MO)

³ Imidacloprid (160 µg / seed), or clothianidin (250 µg / seed), Bayer CropScience, Research Triangle Park, NC

⁴ Lambda-cyhalothrin (Warrior), Syngenta, Greensboro, NC, 11.4% AI, 222 ml/ha

⁵ Rogers Attribute WSS 0984 (Syngenta Seeds, Boise, ID)

Table 2. Species list of Carabidae collected from sweet and field corn in Rock Springs, PA during 2003 and 2004.

<i>Agonum cupripenne</i> Say	<i>Diplocheila obtusa</i> LeConte
<i>A. muelleri</i> Herbst	<i>Dischyrus globulosus</i> Say
<i>A. placidum</i> Say	<i>Elaphropus anceps</i> (LeConte)
<i>A. punctiforme</i> (Say)	<i>Harpalus affinis</i> Shrank
<i>Amara aenea</i> DeGeer	<i>H. caliginosus</i> F.
<i>A. exarata</i> Dejean	<i>H. compar</i> LeConte
<i>A. familiaris</i> Duftschmidt	<i>H. erythropus</i> Dejean
<i>A. impuncticollis</i> Say	<i>H. faunus</i> Say
<i>Anisodactylus harrisi</i> LeConte	<i>H. herbivagus</i> Say
<i>A. rusticus</i> Say	<i>H. longicollis</i> LeConte
<i>A. sancataecrucis</i> (F.)	<i>H. pensylvanicus</i> DeGeer
<i>Bembidion affine</i> Say	<i>H. rubripes</i> Duftschmidt
<i>B. mimus</i> Hayward	<i>Microlestes linearis</i> LeConte
<i>B. quadrimaculatum oppositum</i> Say	<i>M. pusio</i> LeConte
<i>B. rapidum</i> LeConte	<i>Patrobus longicornis</i> Say
<i>Bradycellus rupestris</i> Say	<i>Poecilus chalcites</i> Say
<i>Calathus gregarius</i> Say	<i>P. lucublandus</i> Say
<i>Chlaenius tricolor</i> Dejean	<i>Pterostichus melanarius</i> Illiger
<i>Cicindela punctulata</i> Olivier	<i>P. mutus</i> Illiger
<i>C. sexgattata</i> F.	<i>P. stygicus</i> Say
<i>C. tranquebarica</i> Herbst	<i>Scarites quadriceps</i> Chaudoir
<i>Clivina bipustulata</i> F.	<i>Stenolophus comma</i> F.
<i>C. impressifrons</i> LeConte	<i>S. ochropezus</i> (Say)
<i>Colliuris pensylvanica</i> (L.)	<i>Trechus quadristriatus</i> (Shrank)
<i>Cyclotrachelus furtivus</i> LeConte	

Table 3. Significant results ($P < 0.05$) of proc mixed (SAS v. 9.1.3) repeated measures ANOVA for most abundant Carabidae collected from maize in Rock Springs, PA. Analyses were performed for each species by year and crop and examined the effects of date, transgenic technology (TT), insecticide use (Ins), and their interactions. Date was significant for each species in every crop and year, and therefore not included in the table.

Species	Year	Crop	Effect	F	df	P
<i>S. quadriceps</i>	2003	Sweet Corn	TT	4.15	1,78	0.0450
<i>P. chalcites</i>	2004	Field Corn	TT	10.46	1,86	0.0017
			TT*Ins	9.36	1,86	0.0030
		Sweet Corn	Ins	6.54	1,86	0.0123
			TT*Ins	4.95	1,86	0.0287
<i>P. melanarius</i>	2003	Field Corn	TT*Ins	6.89	1,78	0.0104
		Sweet Corn	TT	9.01	1,78	0.0036
			Ins	10.49	1,78	0.0018
			TT*Ins	9.98	1,78	0.0023
			Date*TT	2.24	9,78	0.0279
			Date*Ins	3.53	9,78	0.0010
			Date*TT*Ins	2.92	9,78	0.0049
<i>H. pensylvanicus</i>	2003	Field Corn	TT*Ins	4.13	1,78	0.0455
			Date*TT*Ins	2.54	9,78	0.0129
	2004	Field Corn	TT	5.01	1,86	0.0278
			Date*TT*Ins	2.01	10,86	0.0419

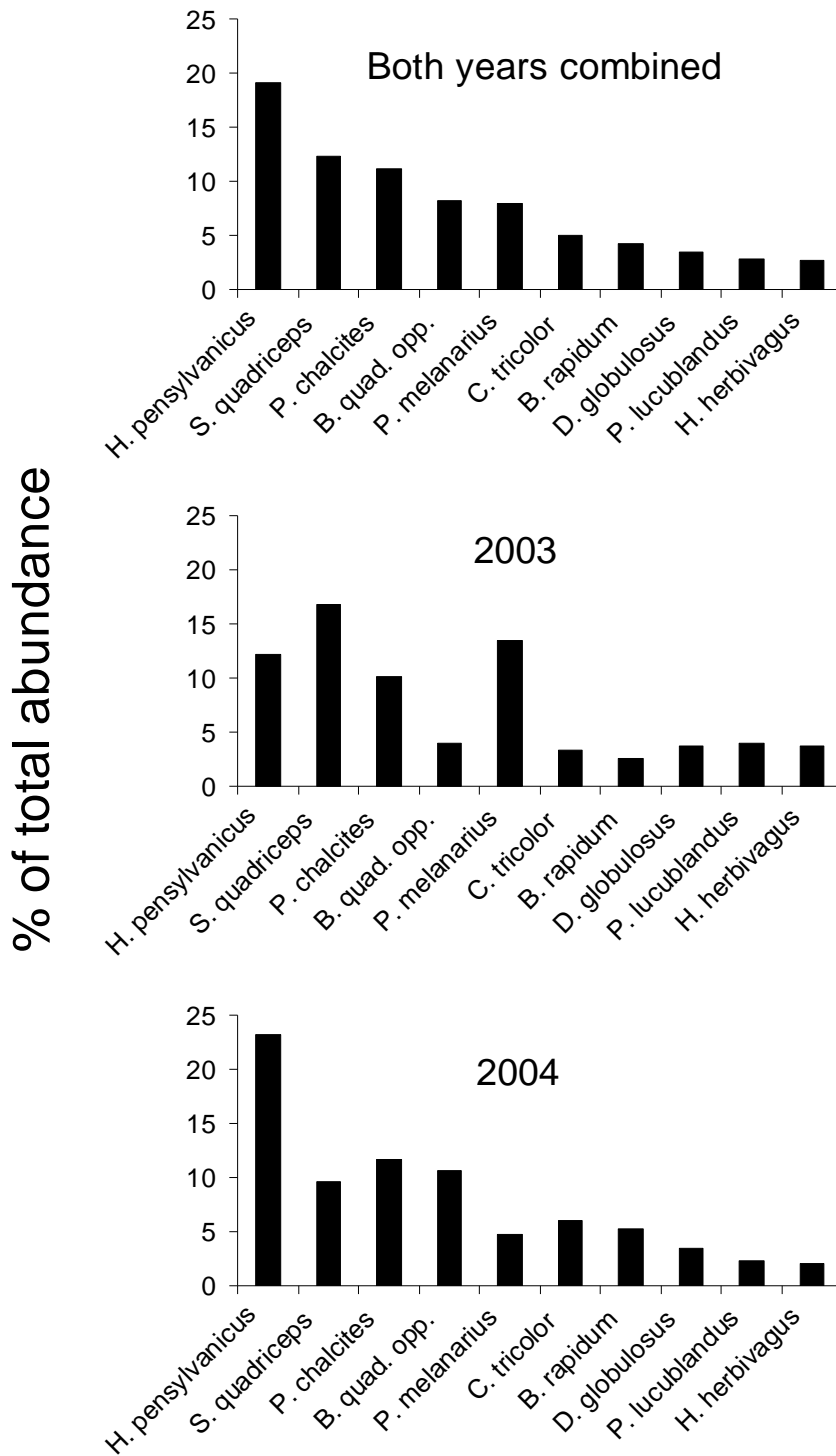


Figure 1. Dominance distribution of the most abundant Carabidae collected in maize in Rock Springs, PA during 2003 (n = 2705 carabids) and 2004 (n = 4535 carabids). Refer to species list (Table 2) for full genus names.

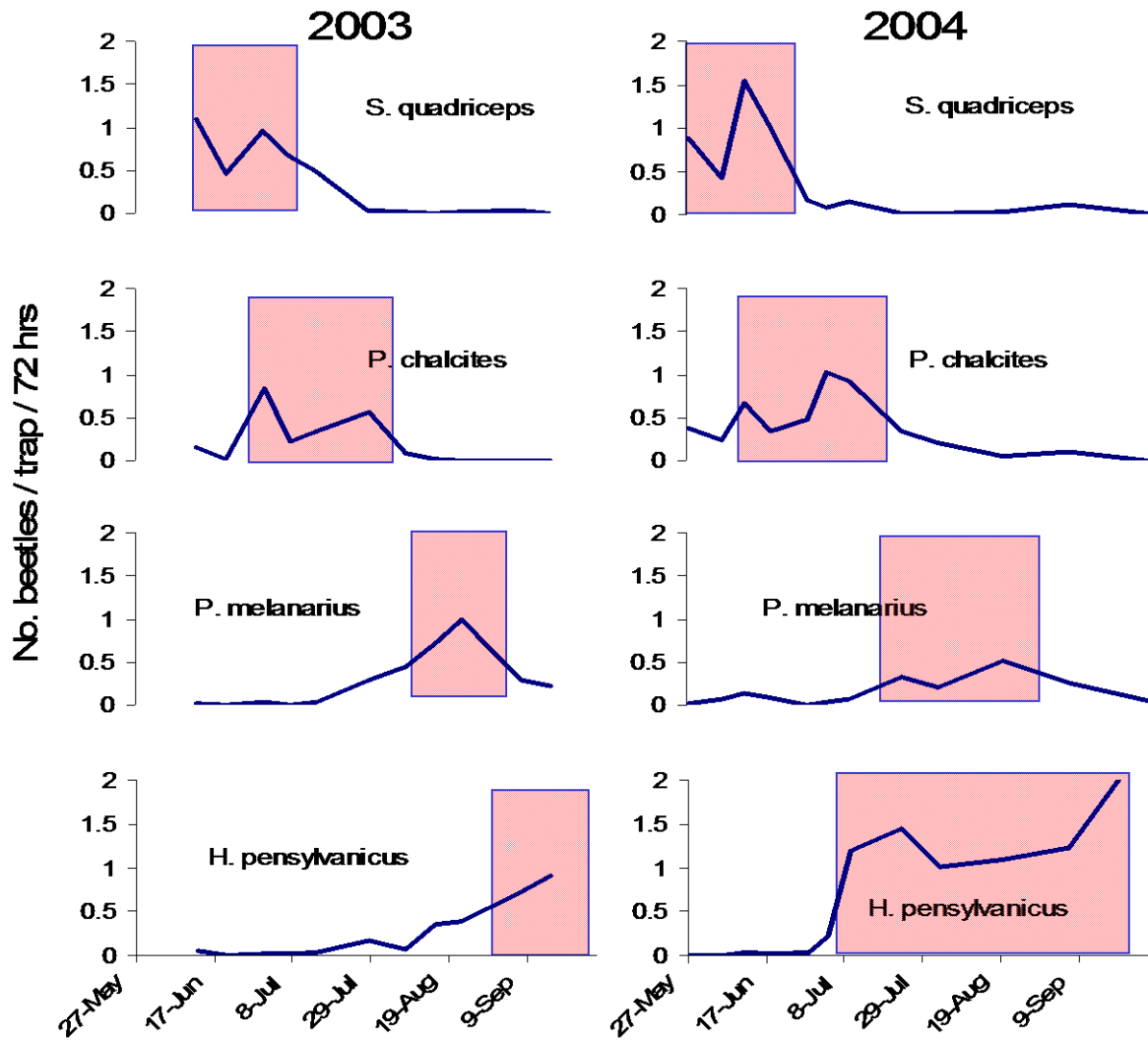


Figure 2. Population dynamics of the four most abundant carabid species in maize at Rock Springs, PA (2003 and 2004). Shaded areas depict time of peak activity densities.

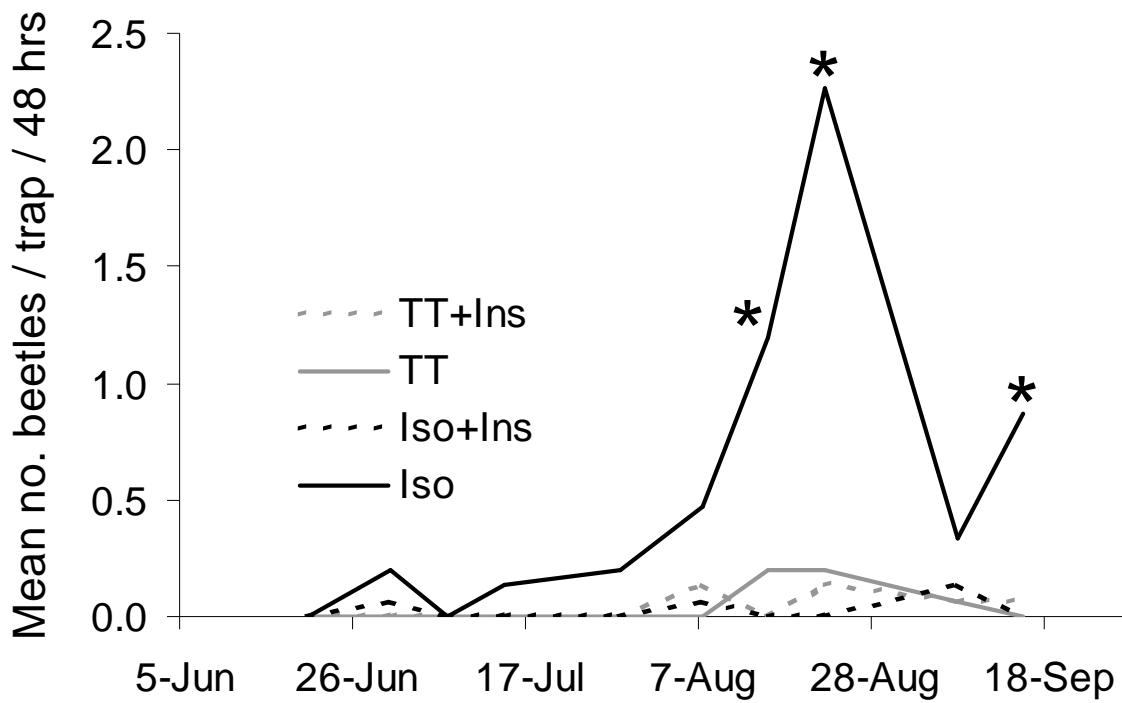


Figure 3. 2003 population dynamics of *P. melanarius* in sweet corn for each of four treatments: 1) transgenic technology (TT), 2) transgenic technology with conventional insecticides (TT + Ins), 3) near isoline with conventional insecticides (Iso + Ins), and 4) near isoline without insecticides (Iso). Asterisks (*) indicate date where activity-density of *P. melanarius* was significantly higher in Isoline / Unmanaged treatment than in other three treatments based on Tukey means separation test ($\alpha = 0.05$).

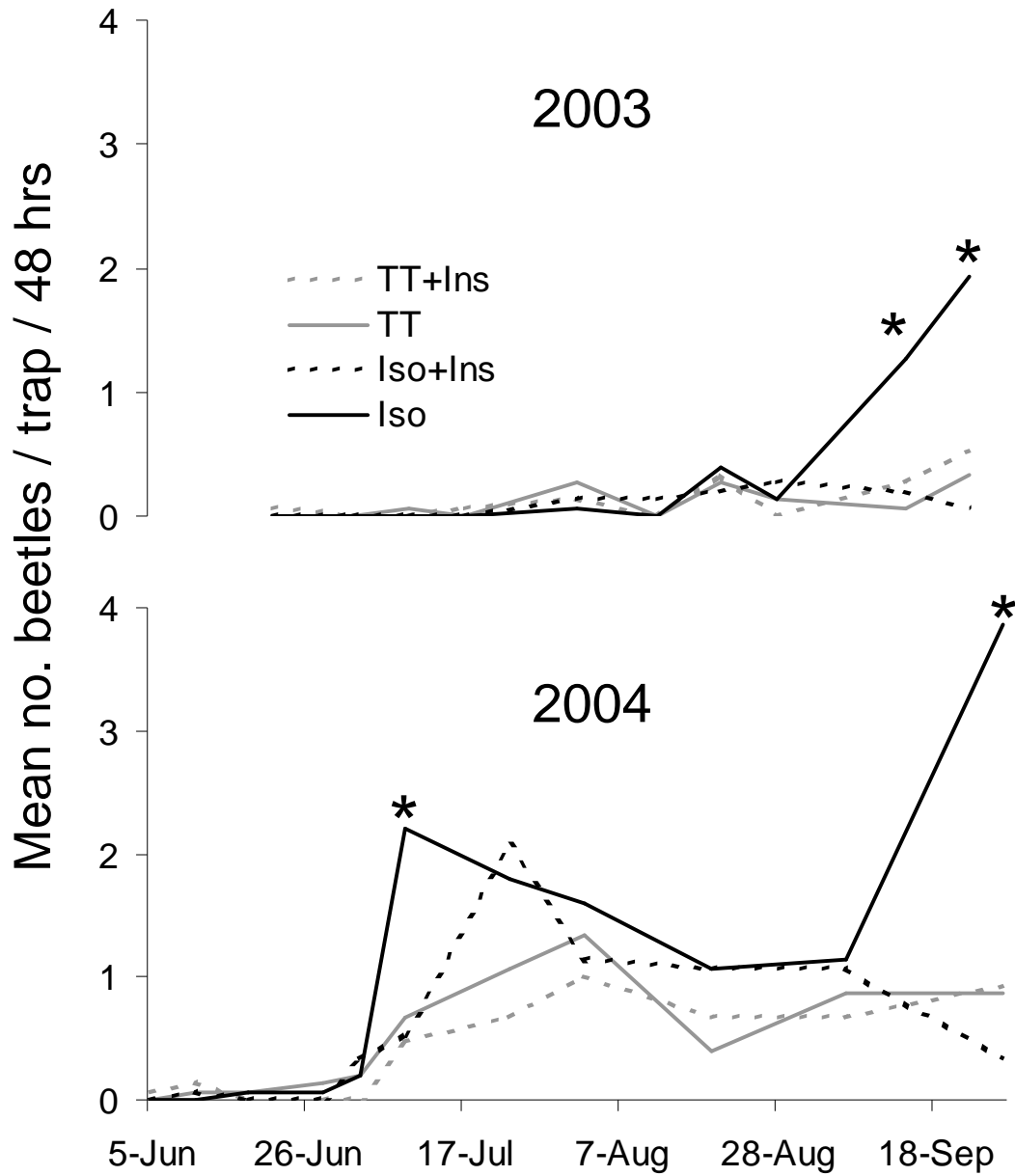


Figure 4. 2003 and 2004 population dynamics of *H. pensylvanicus* in field corn for each of four treatments: 1) transgenic technology (TT), 2) transgenic technology with conventional insecticides (TT + Ins), 3) near isoline with conventional insecticides (Iso + Ins), and 4) near isoline without insecticides (Iso). Asterisk (*) indicates date where activity-density of *H. pensylvanicus* was significantly higher in Isoline / Unmanaged treatment than in other three treatments based on Tukey means separation test ($\alpha = 0.05$).

Chapter 4

Coleopteran Community Dynamics in Mosaics of Transgenic Maize

ABSTRACT

We evaluated non-target coleopteran communities in Cry1Ab/c sweet corn and Cry3Bb field corn. Transgenic management was compared with a control and conventional practices (i.e. insecticide use and crop rotation patterns) currently relevant for each type of maize. Of particular interest was the Cry3Bb field corn, which was not rotated across years and was coupled with neonicotinoid seed treatments. A functionally diverse subset of the coleopteran community consisting of three families – Carabidae (invertebrate and weed seed predators), Chrysomelidae (herbivores), and Nitidulidae (fungivores / detritivores) – representing 9,525 specimens and a diverse array of functional roles, was identified to species and used for statistical analyses. We found no differences in the species richness of Carabidae, the most speciose group, between treatments, however the second year of non-rotated field corn exhibited an increase in carabid activity-density in the control treatment. Using ordination and PERMANOVA, significant community gradients driven by management practices were identified in second year field corn. Communities were largely defined by the control communities differentiating from the treated communities. Results indicate that non-rotated Cry3Bb field corn may have effects on non-target Coleoptera that are indistinguishable from conventional insect control practices, of which neonicotinoid seed treatments or Cry3 proteins may play a role. Crop rotation in the sweet corn seemed to mask treatment effects, identifying crop rotation as a possible species-mixing tool.

INTRODUCTION

Over the past decade, insect pest management in agriculture has shifted dramatically due to the introduction of genetically engineered (GE) crops containing *Bacillus thuringiensis* (*Bt*) transgenes that encode for insecticidal Cry proteins. This pest management approach is considered more localized and/or target-specific than conventional foliar or soil-applied insecticides. Perceived benefits include more efficient control of focal pest taxa, often resulting in less reliance on conventional insecticides (Musser and Shelton 2003), which may promote or sustain on-farm biodiversity (Cattaneo et al. 2006, Leslie et al. 2007). However, concerns regarding negative impacts to non-target insect species from insecticidal GE crops warrant exploration. To date, most evidence suggests few non-target effects (summarized by Romeis et al. 2006), although the need for long-term ecological studies has been highlighted (Shelton et al. 2002).

Transgenic technology has been readily adopted in maize for control of European corn borer (*Ostrinia nubilalis* Hubner) and corn rootworm (*Diabrotica virgifera virgifera* LeConte), utilizing Cry1Ab and Cry3Bb, respectively. Cry1Ab maize, introduced in 1996, and currently commercialized in sweet and field corn cultivars, has been subjected to numerous non-target studies with many concluding that, under field conditions, adverse affects to non-target organisms are non-existent, minimal, or cannot be detected (e.g., Orr and Landis 1997, Pilcher et al. 1997, Wraight et al. 2000, Hoheisel and Fleischer 2007, Leslie et al. 2007), except in instances where the abundance of a specialist predator of the target pest taxa is reduced due to lack of prey (i.e., Pilcher et al. 2005). The more recently developed rootworm-specific Cry3Bb maize, currently

commercialized in field corn cultivars, has directed concerns toward soil and epigeal arthropods, of which initial studies have yet to detect adverse effects (Al-Deeb and Wilde 2003, Ahmad et al. 2005, Bhatti et al. 2005). However, transgenic crops are often coupled with other technologies, such as seed treatments, which may also pose a threat to non-target organisms and should be considered in ecotoxicological studies. In the U.S. today, all available Cry3Bb field corn contains neonicotinoid seed treatments, recently determined to be highly toxic to carabids (Mullin et al. 2006). It is therefore important to assess the impact of not only the transgene, but coupled technologies that are inherently introduced with the transgenic technology.

Field studies examining communities often resort to family-level taxonomic resolution (e.g., Bhatti et al. 2005, Rose and Dively 2007), while those with species-level taxonomic resolution are primarily population studies examining one or a few species (e.g., Lopez et al. 2006). There remains a need for non-target studies assessing effects to communities with species-level taxonomic resolution. The use of taxonomic surrogates in ecotoxicological experiments can result in erroneous conclusions, as higher taxa, especially in insects, can contain numerous species of various sizes and functional roles that may respond differently to the same environmental conditions (Danks 1996). When examining community-level response, species-level identification provides the greatest and most accurate ecological inference, allowing for diversity measurements such as species richness and dominance structure, and the possibility to determine which species are primarily responsible for driving community shifts between treatments.

Additionally, studies with a strong taxonomic investment provide baseline species data

for the area being studied and can be utilized in future studies to examine shifts over longer time periods.

Our study examined the response of coleopteran communities to current insect pest management regimes in Pennsylvania sweet corn and field corn, with a focus on the effects of transgenic technology versus conventional insecticide use. Community dynamics from three Coleopteran families (i.e., Carabidae, Chrysomelidae, Nitidulidae) representing diverse functional roles were analyzed, and all specimens were identified to the species level. Transgenic technology in field corn was of primary concern due to the presence of coleopteran-specific Cry3 proteins and neonicotinoid seed treatments.

METHODS

Experimental design. We used a replicated, split plot design to examine the effects of transgenic technology (transgenic technology vs non-transgenic) and insecticide input (insecticides vs no insecticides) on non-target Coleoptera in sweet corn and field corn. The experiment was conducted on the Penn State University research farms at the Russell E. Larson Agricultural Experimental Station in Pennsylvania Furnace, PA and spanned the 2003 and 2004 growing seasons. The study site contained Hagerstown silt loam soils with 0 to 3 percent slopes (HaA). In each year, we used twelve 1-acre (0.4 ha) experimental units, representing four treatments replicated three times. All experimental units were surrounded with a 3 m alfalfa border. Each experimental unit was comprised of randomly assigned field corn, sweet corn and snap bean subplots (each 55m x 18.3m). Snap beans were present as a rotational crop for sweet corn in the second year, whereas field corn was not rotated. In the year preceding

the experiment, the study site consisted of numerous small plots of a diverse array of crops, including: soybeans, sweet corn, oats, red clover, snap beans, alfalfa, and wheat. No corn followed corn in the first year of our experiment.

The four treatments (detailed in Table 1) were defined by transgenic technology and insecticide management, and can be described as: 1) isoline maize without insecticides (Iso), i.e., control, 2) isoline maize with conventional insecticides (Iso + Ins), 3) Maize utilizing transgenic technology (TT), and 4) maize utilizing transgenic technology with conventional insecticides (TT + Ins)

Insecticide use. In the treatments utilizing conventional insecticides, field corn and sweet corn received an at-planting soil application of the pyrethroid tefluthrin (3.7 kg/ha of Force 3G, Syngenta, Greensboro, NC, 3% AI) for control of corn rootworm and other seed- and root-feeding insects. Additionally, insecticide managed sweet corn received four late-season foliar applications of the pyrethroid lambda-cyhalothrin (222 ml/ha of Warrior, Syngenta, Greensboro, NC, 11.4% AI) for control of lepidopteran ear-feeding pests. These pre-determined insecticide-use patterns were consistent with northeastern maize production.

Transgenic technology. Our choice of transgenes focused on those most likely to influence insecticide use patterns in the U.S. For field corn, we used transgenic cultivars expressing Cry3Bb toxins, aimed at root-feeding stages of *Diabrotica* spp. These Cry3 cultivars are expected to reduce broad-spectrum soil insecticides, but as previously mentioned, these are coupled with neonicotinoid seed treatments to control secondary (non-rootworm) damage to seeds, seedlings, and roots. Specifically, for the field corn transgenic-technology treatment, we used DeKalb DKC60-12 YGRW

(Monsanto Co., St. Louis, MO) field corn expressing Cry3Bb1 endotoxins for control of corn rootworm which contained a neonicotinoid seed treatment [160 µg imidacloprid / seed, or 250 µg clothianidin / seed (Bayer CropScience, Research Triangle Park, NC)] for control of secondary seed- and root-feeding pests. All *Bt* field corn contains neonicotinoid seed treatments, thus this represents realistic evaluation of the influence of “transgenic technology” on coleopteran dynamics. In the field corn experimental units that did not use transgenic technology, we planted the near isoline, DKC60-17, which does not contain a neonicotinoid seed treatment. For transgenic sweet corn we used cultivars expressing Cry1 toxins, aimed primarily at European corn borer, which is expected to reduce broad-spectrum foliar insecticides. Rogers (Syngenta Seeds, Boise, ID) Attribute WSS 0984 sweet corn expressing Cry1Ab/c endotoxins for control of European corn borer was used for the transgenic treatment, while Boreal F1 sweet corn was the near isoline.

The aforementioned insecticide use patterns and transgenic cultivars were also used in the combined treatment (i.e., TT + Ins). Additionally, all corn seed in all treatments was commercially treated with a fungicide blend shown previously not to be directly toxic to Carabidae in the laboratory (details on fungicides and results can be found in Mullin et al. 2005). Herbicide applications were identical between treatments within each year.

Insect collection and identification. Pitfall traps were used to capture all coleopteran specimens. The pitfall traps were made from deli containers, 14.1 cm deep with 10.9 cm inside diameter, and were inserted flush with the ground. Another small collection cup (5.5 cm deep, 8.2 cm inside diameter) filled with ethylene glycol was

placed inside for removal of specimens. An inverted top of a 2-liter soda bottle was put in each deli-container to act as a funnel, limiting escape by beetles and access of the ethylene glycol to small mammals. Ten traps, arranged in two transects of five, were placed at 9, 18, 27, 36 and 45 m within the rows of all field corn and sweet corn subplots for a total of 240 traps. Each subplot of field corn and sweet corn had twenty-two rows and the traps were placed in row numbers eight and fourteen from the south side of the subplot. In 2003, trapping consisted of 11 sampling dates between June 12 – Sept 7. In 2004, there were 12 trapping dates extending from May 27 – Sept 15. Trapping occurred every 7-14 days depending on weather conditions. For each sampling period, one row of five traps was opened in each field corn and sweet corn subplot for a total of 120 open traps. The open rows were alternated each week to avoid over-sampling a portion of the field. All traps were open for 72 hours, after which all beetles were transferred to 70% ethanol. Beetles were later sorted, pinned and identified to species. Identifications were performed by Robert Davidson (Carnegie Museum of Natural History, Pittsburgh, PA), David Biddinger (Penn State Fruit Research and Extension Center, Biglerville, PA) and Tim Leslie (Penn State University, University Park, PA) using several taxonomic keys (Wilcox 1954, Downie and Arnett Jr. 1996, Ciegler 2000, Krinsky and Oliver 2001, Marshall 2006) and voucher specimens from previous studies (Hoheisel 2002, Leslie et al. 2007). Voucher specimens are housed in the lab of S. J. Fleischer, Department of Entomology, Penn State University.

Data analysis. Data were recorded as activity-density (# beetles / pitfall trap / 72 hours) for each species at the subplot-level. We examined species from three families that were abundant in trap captures and represented different functional roles in maize

agroecosystems: 1) Carabidae – carnivores / omnivores / weed seed predators, 2) Chrysomelidae – herbivores, and 3) Nitidulidae – fungivores / detritivores. These coleopteran assemblages were compared between the four treatments by examining species richness using rarefaction, and comparing community composition among treatments, and shifts in this community composition over time, using various ordination methods. We also used a permutational MANOVA (i.e., PERMANOVA; Anderson 2005) to examine the significance of main effects.

We compared species richness between treatments using rarefaction curves generated with EstimateS v. 7.5 (Colwell 2005). Rarefaction curves depict the statistical expectation of species accumulation as sampling effort increases and allows for comparison between treatments with different “sampling efforts” using confidence intervals. Rarefaction curves are generated by iteratively re-sampling the sample-by-species abundance matrix (in which a Monte Carlo permutation procedure randomly re-orders the samples in the matrix). The resulting smoothed species accumulation curve relates cumulative number of species to sampling effort (i.e., number of samples, or number of individuals). Comparing species richness between treatments can then be done at a standardized sampling effort instead of comparing total species richness that could be biased due to unintentional differences in sampling effort (Gotelli and Colwell, 2001). We used Carabidae for the rarefaction analysis, since they represented the most speciose and abundant family of coleopterans collected. Analyses were performed for each crop within each year. Rarefaction curves were individual-based and were generated from 99 permutations of the samples.

A redundancy analysis (RDA) was used to determine the main axes of community variation for the most abundant Coleoptera collected during the study. RDA is a form of constrained ordination, or direct gradient analysis. Similar to principal components analysis (PCA), or unconstrained ordination, which defines the optimal linear predictors of species response data, RDA also fits a linear regression model to the species data where the gradients are limited by the explanatory variables (i.e., in our case, transgenic technology, insecticide use, or their interaction). Thus, the ordination is “constrained” by the pre-determined explanatory variables of the experimental design. The significance of the constrained model is tested through a Monte Carlo permutation test, where the samples are randomly re-ordered in the environmental variable matrix. Under the null hypothesis that the species data is independent of the environmental explanatory variables, the F-statistic calculated from the observed species-environmental variable matrix is compared to the distribution of F-statistics generated from the permuted matrices. Forward selection of the environmental variables can then be used to determine which variables are the best predictors of the species response data.

Using CANOCO 4.5 (ter Braak and Šmilauer 2002), RDAs were performed on the most abundant members (those representing > 2% of total catch of each family in each year) of three coleopteran families (i.e., Carabidae, Chrysomelidae, Nitidulidae). Analyses were performed for each crop in each year. Statistics were generated through 499 permutations of the samples. Bi-plots depicting the first two ordination axes were visualized to identify species’ association with the two explanatory variables and their interactions. We report the significance of the axes and the amount of variation in the species data they explain. All abundance data were Hellinger-transformed (Legendre and

Gallagher 2001) to standardize by sample and account for the numerous cells with zeroes or low numbers. In CANOCO, data were centered and standardized and species scores were divided by the standard deviation after axes extraction. Block was used as a covariable in the analysis.

A permutational multivariate analysis of variance (PERMANOVA) was used to examine the significance of the treatment variables on community composition and abundance. A PERMANOVA tests the response of multiple dependent variables (e.g., species) to multiple treatment variables based on distance measures using permutational methods. This allows for the statistical examination of each treatment variable while accounting for the variation attributable to all the other treatment variables, unlike the stepwise selection procedure in an RDA. Data were normalized using a Bray-Curtis distance measure, a common normalization procedure for ecological data, and permuted 499 times.

Principal response curves (PRC) were developed using CANOCO 4.5 to determine how the coleopteran community responded to treatment variables over time, relative to a control. PRC, like RDA, is a constrained ordination approach; however, the explanatory variables are the interaction of the treatment variables with each sampling point in time – a form of multivariate repeated measures analysis. In our analysis, we identified the isoline maize receiving no insecticides 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 on the most abundant species (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 since the two crops differed in pest management inputs and rotation. As in the RDA, data were Hellinger-transformed (Legendre and Gallagher 2001) prior to 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 in the PRC.

To investigate how closely individual species within the community follow the overall community patterns in each treatment, the PRC procedure provides individual taxon weights. 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. Taxon weights between -0.5 and 0.5 are generally considered non-significant.

RESULTS

Twenty-six families of Coleoptera (Appendix A) were collected from 2,760 pitfall traps during the two-year study. This included 7,256 Carabidae representing 49 species, 1,208 Chrysomelidae representing 34 species, and 1,061 Nitidulidae representing 7 species (Table 2). These three families were chosen for community analyses due to their diversity in functional roles, high levels of abundance, and our ability to identify them to the species level. The remaining families and species are listed in Appendix I.

Due to the high number of individuals and species of Carabidae, individual-based rarefaction curves were generated to compare Carabidae species richness between treatments. All rarefaction curves approached asymptotes (Fig. 1), indicating that the carabid community was sufficiently sampled during the experiment. Based on the confidence intervals, there were no significant differences in rarefied Carabidae species richness between the four treatments in sweet corn or field corn in either year. However, based on the length of the rarefaction curves in second year field corn, the control treatment (denoted “Iso”) exhibited greater overall activity density (i.e., number of individuals collected), at nearly double the amount found in the other treatments.

Multivariate statistical analyses were performed on the most abundant species from each of the three families, defined as those representing > 2% of total abundance in each year (See Table 2). An ordination of the species data constrained by the treatment variables, or an RDA, identified significant community gradients in second year field corn (Figure 2; 1st axis: $F=1.98$, $P=0.006$; 2nd axis: $F=1.56$, $P=0.018$) and first year sweet corn (Figure 3; 1st axis: $F=2.51$, $P=0.002$; 2nd axis: $F=1.65$, $P=0.012$). In both cases the 1st axis was associated with Iso separating from TT. When looking at the interactions that defined our four treatments (refer to Table 1), this separation was largely driven by the control (denoted “Iso * No Ins” in Figs. 2 and 3) differentiating from the other three treatments. In fact, when using forward selection, the control treatment was identified as a significant treatment factor for both second year field corn ($F = 2.16$, $P = 0.03$) and first year sweet corn ($F = 3.11$, $P = 0.01$). The 2nd axis in the bi-plots was associated with differences between insecticide use and no insecticide use (Figs. 2 and 3).

The ordination bi-plots (Figs. 2 and 3) can be used to determine species groupings and their association with treatment variables. Species shown in the biplots are those that accounted for the greatest differences between communities in the different treatments. Among chrysomelids, the herbivores, in field corn in 2004, *Chaetocnema cribifrons* and *C. pulicaria*, were associated with the isoline treatment, with *C. cribifrons* favoring no insecticide use. Except for *Harpalus herbivagus*, the carabids *Poecilus chalcites*, *Bembidion rapidum*, *Bembidion quadrimaculatum oppositum*, and *Dischyrius globulosus* were negatively associated with insecticides. In contrast to the carabids, the fungivore/detritivore nitidulids *Glischrochilus fasciatus*, *Glischrochilus quadrisignatus*, and *Stelidota geminata* tended to be associated with insecticide use, except for *Carpophilus lugubris* which was found more often in the isoline field corn.

For sweet corn in 2003 (Fig. 3), the most influential species consisted of primarily Carabidae; six of the seven species shown, all except *C. cribifrons*, were Carabidae. All of these influential Carabidae tended to be negatively associated (i.e., vectors point in opposite direction of “Ins”) or uncorrelated (i.e., vectors at right angle to “Ins”) with insecticide use. *P. chalcites*, *P. melanarius* and *P. lucublandus* were found more often in isoline sweet corn, while *H. pensylvanicus*, *S. quadriceps*, and *C. tricolor tricolor* were found more often in transgenic sweet corn. *C. cribifrons*, a chrysomelid, was the only species associated with insecticide use. No significant axes were found in 2003 field corn or 2004 sweet corn.

A PERMANOVA was used to examine main effects (and their interactions) on the coleopteran communities in second year field corn and first year sweet corn (i.e., those crops and years with significant community gradients). Transgenic technology

($F_{1,11}=2.88$, $P=0.02$), insecticide management ($F_{1,11}=2.30$, $P=0.03$) and their interaction ($F_{1,11}=2.1$, $P=0.036$) influenced coleopteran communities in second year field corn. No treatment effects were significant for first year sweet corn, despite the presence of significant axes found in the RDA.

Principal response curves showed a primarily negative response of the treatment coleopteran communities compared 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 community measurements were taken after the first potential effect from either soil insecticides or neonicotinoid seed treatments in TT. At the first measure in June of 2003, we can already see TT differing from the control. By July all three treatment communities differ from the control, which was graphically represented by a steep decline in the treatment communities in July and August, and then a return toward the 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 rebound completely to baseline level as in the previous year.

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, the treatment communities all hovered around the control community with no clear deviation from the control.

Species weights indicated which species followed (species weight > 0.5) or deviated (species weight < -0.05) from the principal response. In field corn, two chrysomelids (*C. cribifrons* and *C. pulicaria*) and three carabids (*P. chalcites*, *P.*

lucublandus, and *B. rapidum*) had species weights above 0.5, while three carabids (*C. tricolor tricolor*, *D. globulosus*, and *S. quadriceps*) and one nitidulid (*S. geminata*) had negative species weights below -0.5.

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

DISCUSSION

Among the variety of approaches for examining the non-target effects of insecticidal transgenic (*Bt*) crops, there remains a need for studies assessing non-target communities with species-level resolution. While species-level identification often requires large investments of time and money, which can be exacerbated by the bottleneck in taxonomic expertise (Kim and Byrne 2006), we propose an approach that focuses on a functionally diverse subset of the non-target community in question, for which species-level taxonomic resolution can be achieved (also see Leslie et al. 2007) and which can reasonably be presumed to be at risk from direct or indirect effects (e.g., coleopterans where Cry3Bb proteins are deployed). In this study we examined the influence of the deployment of *Bt* sweet corn and *Bt* field corn as compared to conventional insect pest management practices on non-target Coleoptera. We chose three families – Carabidae, Chrysomelidae, and Nitidulidae – that were collected in high activity-densities in our traps, represented a diverse range of functional roles, and for which species-level identification could be achieved in a manageable time frame.

To assess the influence of transgenic technology on these coleopteran assemblages in sweet corn and field corn, we compared four treatments defined by the presence or absence of transgenic technology and the presence or absence of conventional insecticides. Although treatments were identical in name, and relevant to current management systems, for both sweet corn and field corn, insect pest management inputs varied greatly (Table 1) based on common management practices and needs in the two systems. Most notably, transgenic field corn expressed coleopteran-specific Cry3-based proteins, while transgenic sweet corn expressed Cry1-based proteins. Additionally, transgenic field corn was coupled with neonicotinoid seed treatments, while transgenic sweet corn did not contain any insecticidal seed treatment. In treatments with conventional insecticides, field corn received an at-planting application of a soil-directed pyrethroid, whereas sweet corn received the at-planting soil pyrethroid and four additional foliar pyrethroid sprays during tasseling and silking for control of ear-damaging insect pests. Lastly, field corn was not rotated between the two years, whereas sweet corn was grown in a sweet corn / snap bean rotation. Due to the management differences and possible subsequent direct and indirect effects to non-target coleopteran communities, sweet corn and field corn were treated separately in analyses.

Field Corn. Field corn was of primary interest in this study due to the presence of the coleopteran-specific Cry3Bb proteins in the transgenic treatment coupled with the neonicotinoid seed treatment technology both of which could present direct effects on Coleoptera. 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. 2006), where the seed treatment, and not the Cry protein, was found to be a significant mortality factor for carabids. Thus, ecologically relevant field assays are warranted.

The fact that field corn was not rotated throughout the two-year experiment made the second year field corn the most reliable indicator of treatment effects. Due to the large size of the experiment, the plots were initially situated over a previously diverse crop mosaic from the year preceding the experiment, of which confounding community legacy effects may have influenced the first year of the experiment. In the second year, community legacy from previous land use was of less concern since field corn treatments remained in the same location for both years allowing coleopteran communities to establish. Indeed, the second year field corn showed indications that coleopteran communities were affected by both transgenic and conventional management inputs. These trends were often distinguished by communities in the control treatment differing from those found in the pest management treatments.

When examining species richness of Carabidae, no significant differences were found. In a similar study in the same region, differences in carabid species richness were also not detectable (Leslie et al. 2007). Although, several studies show impacts of insecticides on carabid diversity (Andersen and Eltun 2000, Hokkanen and Holopainen 1986), the heterogenous habitat in Pennsylvania agroecosystems may be conducive to recolonization after pest management events. One noticeable difference from the rarefaction curves was the length of the control treatment curve in field corn 2004. Despite the lack of differences in species richness, overall activity-density of carabids

(total # of individuals collected) in the 2004 control treatment was noticeably higher than the other treatments and the control community in 2003. Therefore, by the second year of the study, carabid activity-density was reduced in plots receiving a soil insecticide application or containing a neonicotinoid seed treatment (or both).

Ordination analyses indicated the presence of distinct community gradients among the coleopteran community in the second year. The primary gradient (Fig. 2, 1st axis) was associated with the control differentiating from the other treatments, for which a clear distinction can be seen in the PRC (Fig. 5). Most species were associated with the isoline treatments. The herbivores (*C. cribifrons*, *C. pulicaria*, *H. herbivagus*) may have responded negatively to the systemic neonicotinoid, or possibly the Cry3 toxins.

Harwood et al. (2005) detected *Bt* Cry1Ab endotoxins in non-target coleopteran herbivore species, including *C. pulicaria*, taken from *Bt* maize fields. It is yet unclear if non-target chrysomelids could be affected by the uptake of Cry3Bb endotoxins directed at *Diabrotica* spp. One of the larger carabids, *P. chalcites*, was found more often in the control, as well the fungivore / detritivore *C. lugubris*. While *P. chalcites*, may have exhibited greater survival in control plots due to the lack of insecticides, *C. lugubris* (dusky sap beetle), a known pest of maize, may have been found in greater numbers because it was attracted to insect damage (not quantified) in the unmanaged corn. Other nitidulid detritivores, *S. geminata* (strawberry sap beetle) and *G. quadrisignatus* (picnic beetle), were found more often in the treatments with insecticides but may not be as intimately associated with maize as *C. lugubris*. Two carabids, *D. globulosus* and *B. quadrimaculatum oppositum*, deviated from the majority of the community and were found more often in the transgenic treatment, while *P. chalcites* and *B. rapidum* were

found more often in the isoline treatment. All four species responded negatively to soil applied pyrethroid insecticides, despite their difference between transgenic and isoline maize.

When examining these community shifts over time using PRC, it became apparent that the non-target community responded negatively to the pest management inputs, as compared to the control (Fig. 4). Interestingly, this includes the transgenic technology treatment, which in addition to expressing Cry3Bb toxins, only contained a neonicotinoid seed treatment and did not receive any soil insecticides. Bhatti et al. (2005) considered the effect of seed treatments and soil and foliar-applied insecticides on non-target Coleoptera, and although results varied, they found significantly lower abundances of Carabidae and Nitidulidae, in the seed treatment or soil-insecticide treatments as compared to a control in at least one year of the experiment. This same study found relatively few differences between communities collected from *Bt* (Cry3Bb) and non-*Bt* field corn. In laboratory bioassays, Mullin et al. (2006) identified neonicotinoid seed treatments found on Cry3Bb field corn as a major direct mortality factor for carabids, and not the Cry3Bb endotoxins.

In what seems to be a response to the at-planting pest management inputs in late May / early June, the principal response for the treatment communities noticeably drops in relation to the control (Fig. 4). By September, the treatment communities reconvene near the control community. This may indicate a recovery process in the field, or the natural temporal succession of species may slowly show a lack of treatment effect as new species emerge. Individual taxon weights indicated similar trends as found on the ordination bi-plot; the chrysomelids, the dusky sap beetle, and several carabids closely

followed the principal response. As in the bi-plot, *S. geminata*, responded in an opposite fashion and several carabids also had negative taxon weights. One of the carabids with a strong negative taxon weight, *C. tricolor tricolor*, was much more abundant in the second year and may have been filling the empty niche created by the decline of some species in the treated plots.

Sweet Corn. Gradients in the sweet corn coleopteran communities were detectable in the first year of the study, possibly a reflection of the communities defined by the diverse crop mosaic in the year preceding the experiment (i.e., legacy effect). PERMANOVA indicated that these gradients were not significantly associated any of the treatment variables in this 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). Since land use in the sweet corn plots was not continuous between any two years, treatment effects should possibly be tested further in a multi-year experiment where coleopteran communities are able to establish. However, it is worth noting that vegetable crops, such as sweet corn, are commonly rotated in commercial production systems.

The community gradients found in 2003 were largely defined by carabids. Association with treatments varied by species, however all were either uncorrelated or negatively correlated to insecticide use. Only, *C. cribifrons*, a chrysomelid, was found to positively associate with insecticide use. No significant community gradients were found in the second year. When examining community shifts over time with PRC, treatment effects were again detectable only in 2003. Similar to field corn in 2004, the coleopteran communities initially responded negatively to any of the seed or soil treatments, yet these

communities appeared to have reconvened by the fall of the treatment year. The negative response to the transgenic sweet corn is difficult to explain, as transgenic sweet corn did not contain neonicotinoid seed treatments like the transgenic field corn, and all tests to date have not found any negative effects from Cry1Ab proteins on non-target coleopterans. In the second year, after rotation, all communities on the PRC hover near the control with no distinct trend for community effects. While rotation behind the same snap bean crop seems to have removed all treatment effects, it was surprising to find community patterns in the first year and such results may have been largely driven by community legacy from variation in previous land use.

As with field corn, no significant difference in species richness was found between treatments in either year. A noticeably higher number of carabids were caught in the control treatment of sweet corn in the first year. Whether this is reflecting a reduction of carabids in the treated plots is debatable as the trend was not seen in the second year. Likewise, the control community reached a species richness asymptote more rapidly than the treated communities in the first year, however the trend was reversed in the second year.

Conclusions. Species-level identifications in ecotoxicological studies can provide a more ecologically-relevant evaluation of community response, which may be lost when using taxonomic surrogates. Although initial investment of time and monetary resources for species-level identifications can be substantial, subsequent years of study become easier due to the development of voucher collections and increased taxonomic expertise or established taxonomic collaborations.

This study indicates that non-target coleopteran communities can respond to both conventional and transgenic technology management practices in maize. This was especially evident in the second year of non-rotated field corn where communities were able to establish, and were exposed to two years of the same treatments in the same location. Although longer-term studies are needed, early indications are that non-target herbivores may be affected by Cry proteins coupled with systemic neonicotinoids found on transgenic field corn seed, or by pyrethroids applied to soil at planting. Results for carabids and nitidulids varied between species, however the tendency was for higher activity-densities in the control treatment.

PRC analyses suggested that transgenic and conventional management effects on non-target coleopterans were indistinguishable, yet both showed a transitory deviation from the untreated control. This is most likely due to the presence of the neonicotinoid seed treatment associated with the transgenic seed, since there is no evidence that Cry proteins are directly affecting non-target organisms, although non-target chrysomelids are known to uptake Cry toxins from transgenic maize agroecosystems. Our work suggests coleopteran community-level effects are more apparent after multiple years of non-rotated management; thus these effects from systems using coupled transgenic-neonicotinoid technology warrant further study in longer-term experiments.

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Table 1. Pest management inputs defining the experimental treatments for two types of maize.

Treatment	Type of maize	
	Field Corn	Sweet Corn
Control	None	None
Conventional insecticides	Pre-emergence soil insecticide ¹	Pre-emergence soil insecticide ¹ Foliar applications during silking growth stage ⁴
Transgenic technology	Cry3Bb1 toxins ² Neonicotinoid seed treatment ³	Cry1Ab/c toxins ⁵
Conventional insecticides and transgenic technology	Pre-emergence soil insecticide ¹ Cry3Bb1 toxins ² Neonicotinoid seed treatment ³	Pre-emergence soil insecticide ¹ Foliar applications during silking growth stage ⁴ Cry1Ab/c toxins ⁵

¹ Tefluthrin (Force 3G), Syngenta, Greensboro, NC, 3% AI, 3.7 kg/ha

² DeKalb DKC60-12 YGRW (Monsanto Co., St. Louis, MO)

³ Imidacloprid (160 µg / seed), or clothianidin (250 µg / seed), Bayer CropScience, Research Triangle Park, NC

⁴ Lambda-cyhalothrin (Warrior), Syngenta, Greensboro, NC, 11.4% AI, 222 ml/ha

⁵ Rogers Attribute WSS 0984 (Syngenta Seeds, Boise, ID)

Table 2. Species list for three coleopteran families collected at Penn State Research Farms in Rock Springs, PA during 2003 and 2004.

CARABIDAE	CARABIDAE cont.	CHRYSOMELIDAE cont.
<i>Agonum placidum</i>	<i>Harpalus herbivagus*</i>	<i>Distigmoptera apicalis</i>
<i>Agonum punctiforme</i>	<i>Harpalus longicollis</i>	<i>Epitrix cucumeris</i>
<i>Agonum cupripenne</i>	<i>Harpalus pennsylvanicus*</i>	<i>Epitrix fuscula</i>
<i>Agonum muelleri</i>	<i>Harpalus rubripes</i>	<i>Epitrix hirtipennis</i>
<i>Amara aenea</i>	<i>Microlestes linearis</i>	<i>Epitrix humeralis</i>
<i>Amara exrata</i>	<i>Microlestes pusio</i>	<i>Hornaltica atriventris</i>
<i>Amara familiaris</i>	<i>Patrobis longicornis</i>	<i>Lema trilineata</i>
<i>Amara impuncticollis</i>	<i>Poecilus chalcites*</i>	<i>Longitarsus melanurus</i>
<i>Anisodactylus sancataecrucis</i>	<i>Poecilus lucublandus*</i>	<i>Longitarsus subrufus</i>
<i>Bembidion affine</i>	<i>Pterostichus melanarius*</i>	<i>Longitarsus succineus</i>
<i>Bembidion minus</i>	<i>Pterostichus stygicus</i>	<i>Longitarsus waterhousi</i>
<i>Bembidion quadrimaculatum*</i>	<i>Scarites quadriceps*</i>	<i>Mantura chrysantheni</i>
<i>Bembidion rapidum*</i>	<i>Stenolophus comma</i>	<i>Odontota dorsalis</i>
<i>Bradycellus rupestris</i>	<i>Stenolophus ochropezus</i>	<i>Ophraella conferta</i>
<i>Calathus gregarius</i>	<i>Trechus quadristriatus</i>	<i>Phyllotreta striolata</i>
<i>Chlaenius tricolor tricolor*</i>		<i>Phyllotreta zimmermanni</i>
<i>Cicindela punctulata</i>	CHRYSOMELIDAE	<i>Psylloides convexior</i>
<i>Cicindela sexgattata</i>	<i>Anisostena nigrata</i>	<i>Psylloides punctulata</i>
<i>Cicindela tranquebarica</i>	<i>Chaetocnema confinis</i>	<i>Systema elongata</i>
<i>Clivina bipustulata</i>	<i>Chaetocnema cribrifrons*</i>	<i>Systema frontalis</i>
<i>Clivina impressifrons</i>	<i>Chaetocnema denticulata</i>	<i>Systema hudsonias</i>
<i>Colliuris pensylvanica</i>	<i>Chaetocnema minuta*</i>	
<i>Cyclotrachelus furtivus</i>	<i>Chaetocnema pulicaria*</i>	NITIDULIDAE
<i>Dischyrius globulosus*</i>	<i>Chalepus dorsalis</i>	<i>Carpophilus brachypterus</i>
<i>Elaphropus anceps</i>	<i>Diabrotica undecimpunctata</i>	<i>Carpophilus lugubris*</i>
<i>Harpalus affinis</i>	<i>Diabrotica virgifera</i>	<i>Glischrochilus fasciatus*</i>
<i>Harpalus caliginosus</i>	<i>Disonycha collata</i>	<i>Glischrochilus quadrasignatus*</i>
<i>Harpalus compar</i>	<i>Disonycha triangularis</i>	<i>Stelidota geminata*</i>
<i>Harpalus erythropus</i>	<i>Disonycha xanthomelas</i>	

* denotes most abundant species in each family (those representing > 2% of total abundance for each family)

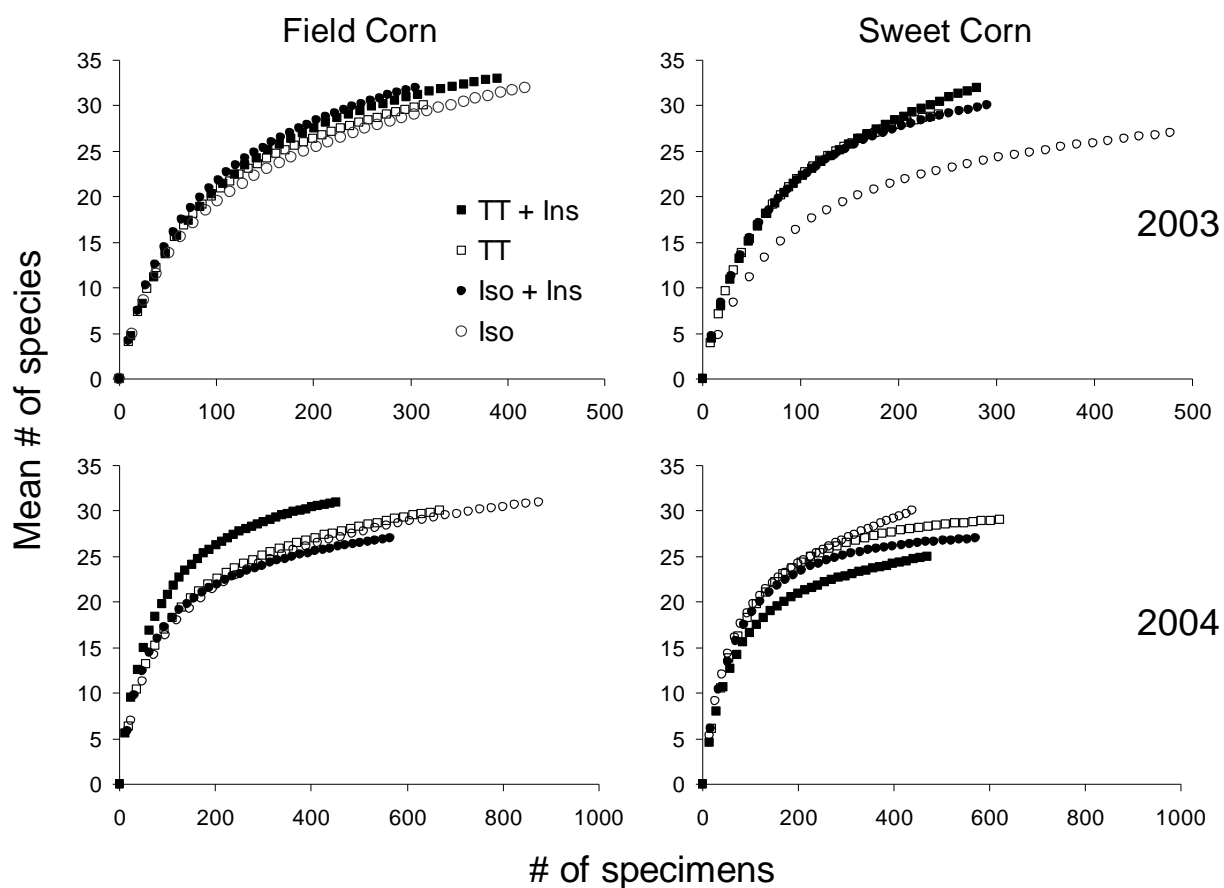


Figure 1. Individual-based rarefaction curves showing Carabidae species accumulation in field corn and sweet corn in 2003 and 2004. Four treatments defined as: isoline corn without insecticides (Iso, i.e. control), isoline corn with conventional insecticides (Iso+Ins), transgenic technology (TT), and transgenic technology with conventional insecticides (TT+Ins).

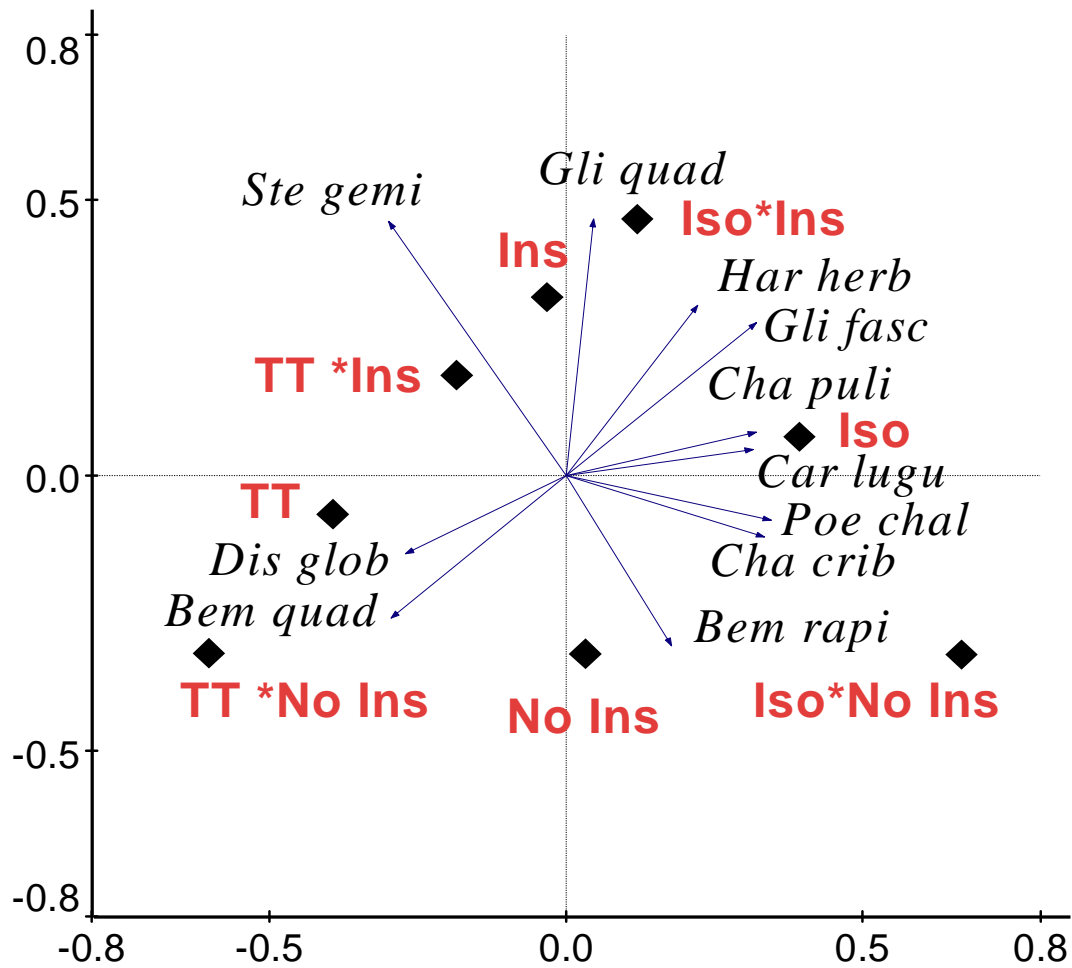


Figure 2. RDA bi-plot showing relative associations between species (arrows) and treatment variable (diamonds) in field corn in 2004. Treatment variables include: 1) transgenic technology (TT) vs near isoline (Iso), 2) conventional insecticides (Ins) vs no insecticide use (No Ins) and 3) all interactions. Species shown are those most influenced by treatment variables. Species are indicated by the first three letters of the genus and first four letters of the species epithet. See Table 2 for complete list and names of species included in the analysis (those denoted with an asterisk, *).

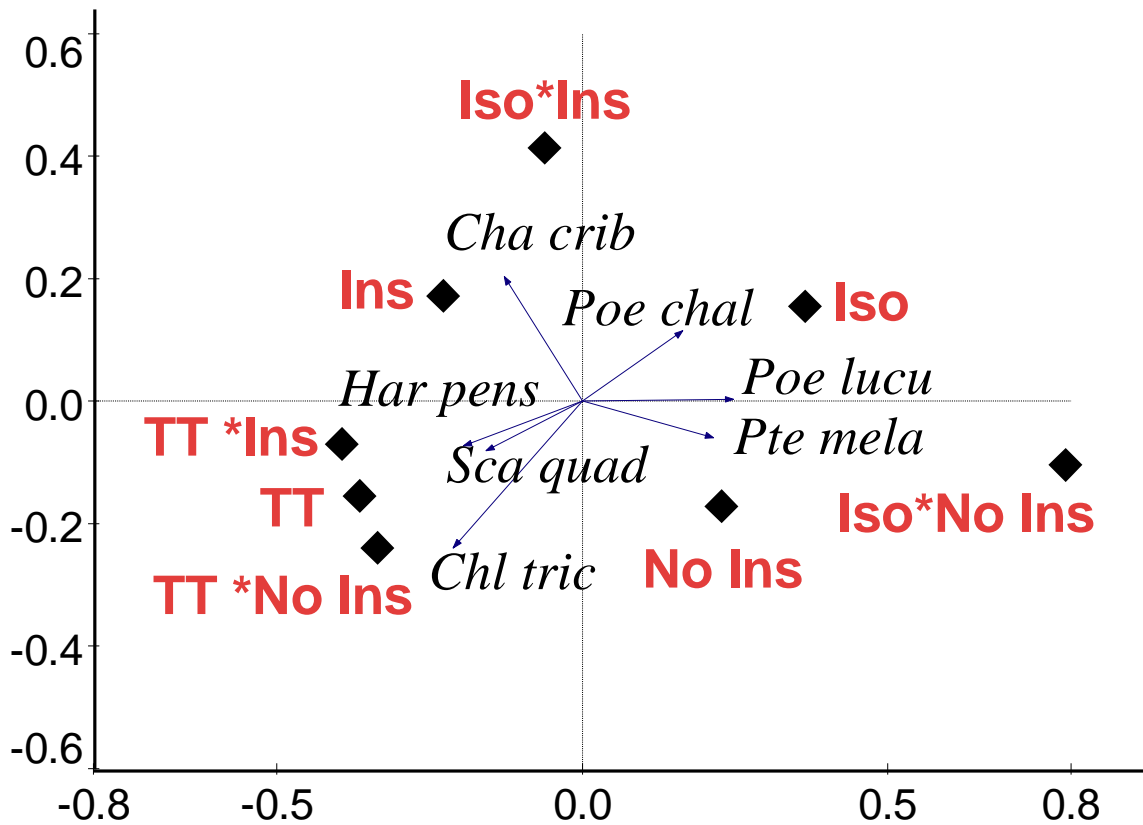


Figure 3. RDA bi-plot showing relative associations between species (arrows) and treatment variable (diamonds) in sweet corn in 2003. Treatment variables include: 1) transgenic technology (TT) vs near isoline (Iso), 2) conventional insecticides (Ins) vs no insecticide use (No Ins) and 3) all interactions. Species shown are those most influenced by treatment variables. Species are indicated by the first three letters of the genus and first four letters of the species epithet. See Table 2 for complete list and names of species included in the analysis (those denoted with an asterisk, *).

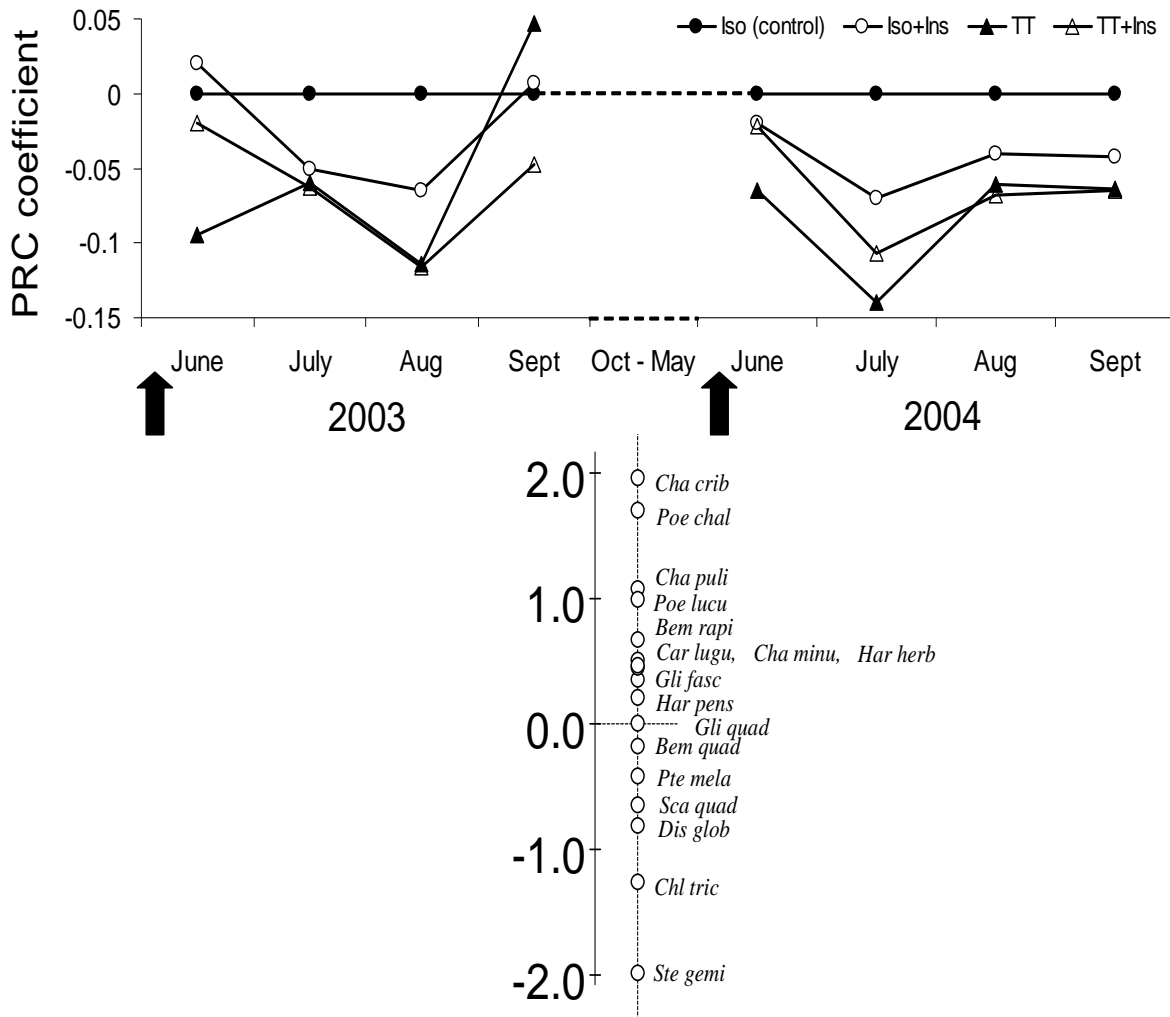


Figure 4. Principal response curves and taxon weights indicating response of non-target coleopteran species to three insect pest management strategies of field corn over two years in relation to a control (shown as horizontal 0.0 line on figure). Treatments were defined as: isoline corn without insecticides (Iso, i.e. control), isoline corn with conventional insecticides (Iso+Ins), transgenic technology (TT), and transgenic technology with conventional insecticides (TT+Ins). Species with positive species weights (> 0.5) follow the principal response, whereas species with negative species weights (< -0.5) responded in the opposite fashion. Arrows indicate when insect pest management inputs occurred (see Table 1 for details). Species are indicated by the first three letters of the genus and first four letters of the species epithet. See Table 2 for names of species included in the analysis (those denoted with an asterisk, *).

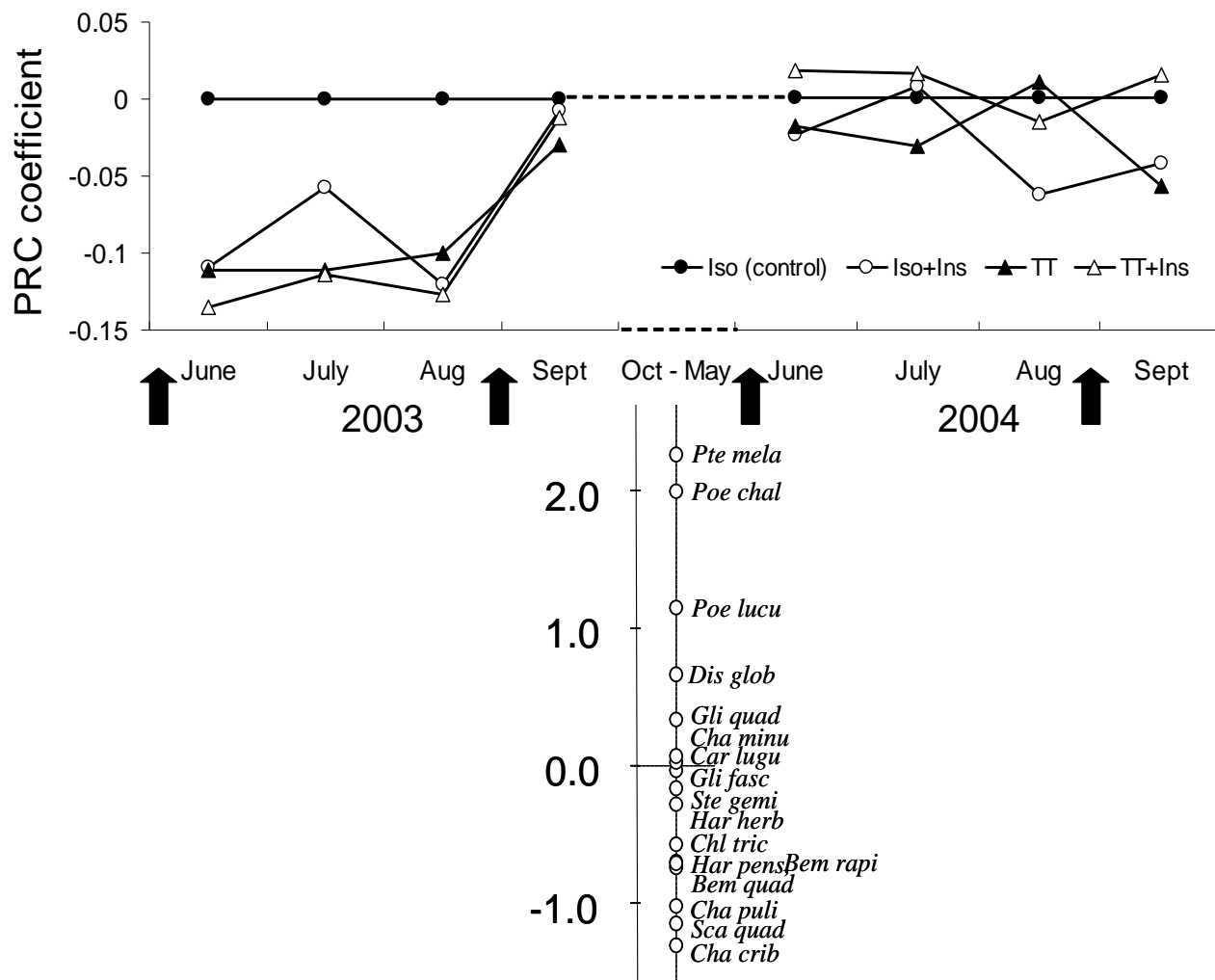


Figure 5. Principal response curves and taxon weights indicating response of non-target coleopteran species to three insect pest management strategies of sweet corn over two years in relation to a control (shown as horizontal 0.0 line on figure). Treatments were defined as: isoline corn without insecticides (Iso, i.e. control), isoline corn with conventional insecticides (Iso+Ins), transgenic technology (TT), and transgenic technology with conventional insecticides (TT+Ins). Species with positive species weights (> 0.5) follow the principal response, whereas species with negative species weights (< -0.5) responded in the opposite fashion. Arrows indicate when insect pest management inputs occurred (see Table 1 for details). Species are indicated by the first three letters of the genus and first four letters of the species epithet. See Table 2 for names of species included in the analysis (those denoted with an asterisk, *).

Chapter 5

Carabidae community dynamics across an ag-forest ecotone

ABSTRACT

We used transects of pitfall traps extending from a cornfield, through a mowed grassy margin, and into an adjacent forest to investigate shifts in carabid community composition across ecotones. Within the corn, we also compared carabid communities between transgenic technology (Cry3Bb corn with neonicotinoid seed treatment) and conventional management (at-planting soil pyrethroid application) practices. Plant diversity and structure measurements were recorded as explanatory variables at each trap. Communities exhibited the highest levels of species richness and evenness in the forest edge, which was positively correlated to plant diversity measures. No differences in carabid diversity were detected between insect pest management tactics in corn. Ordination identified forest as the primary factor defining community differences, and habitat generalists and specialists were identified. Distribution of abundances along the transect indicated that no species were confined to a single habitat. A novel use of principal response curves depicted the greatest amount of community change occurring in the intermediate grassy margin. Based on similarity indices and community composition, the carabid community in forest-edge corn differed substantially from carabid communities monitored in other corn fields not adjacent to forest. We concluded that a forested margin can influence the carabid diversity within neighboring agricultural fields, and future studies are needed to determine if this translates into greater diversity and ecosystem function.

INTRODUCTION

Insects are often used as environmental, ecological or biodiversity indicators because of their ubiquity and importance in the functioning of ecosystems (McGeogh 1998, Büchs 2002). Despite the well-known reliability of aquatic invertebrates as indicators of stream health (EPA 2007), terrestrial insects have had varying degrees of bio-indicative success, often due to issues in scaling (McGeogh 1998). In agricultural settings, beneficial insects comprising functional roles, such as predators, parasitoids, and pollinators, are often monitored to assess impacts from agricultural inputs (Büchs 2002) or to infer the stability and diversity of an agroecosystem (Duelli et al. 1999, Sommaggio 1999, Burgio and Sommaggio 2007). Although many agricultural practices can have direct and indirect effects on non-target insect communities in crop fields, these communities can also be influenced by the surrounding landscape (Marino and Landis 1996, Ovenden et al. 1998), especially habitat directly adjacent to the crop (Holland and Fahrig 2000, Ponti et al. 2005). It is therefore important to consider edge effects when conducting experiments intended to monitor within-field processes.

Among the farm management inputs that may directly or indirectly affect non-target organisms and diversity, few have received as much attention as genetically modified crops. Over the last decade, adoption rates of insecticidal *Bt* crops have steadily risen in U.S. agriculture (USDA 2006, NASS 2006) and numerous non-target studies have been conducted (reviewed by Romeis et al. 2006). While most studies have concluded that effects are non-existent or small, in instances where *Bt* technology is coupled with other insect control methods, such as neonicotinoid seed treatments, non-target effects may be similar to those from conventional insect control practices (Chapter

3 in this thesis). The detectability of such non-target effects may be influenced by insect community legacy from crop rotations (Leslie et al. 2007) and/or plant diversity on the farm and the surrounding habitat (Purtauf et al. 2005). This is especially true for northeastern U.S. agroecosystems, which are often small in size, diversified, and embedded in heterogeneous environments, thereby creating an abundance of edges. For example, in Pennsylvania approximately 60% of the land is forested and many agricultural fields can be found directly adjacent to large acreages of forested land.

The integration of landscape ecology with conventional agronomy redefined the contribution of field margins from merely delineating features to important bio-reserves that could influence the within-field dynamics of agriculture (Thomas & Marshall 1999, LeCoeur et al. 2002). Field margins may vary greatly in floral and faunal composition yet, in an ecological context, all are inevitably intertwined with the neighboring agricultural fields (Marshall & Moonen 2002). These adjacent habitats have many beneficial aspects in agriculture; they can act as reserves for natural enemies and beneficial arthropods, prevent soil and nutrient loss, provide windbreaks and suppress weed, virus and insect pest invasion (Altieri and Nicholls 1999). Conversely, they can also harbor polyphagous pests or serve as reservoirs for pathogens; for example, virus-transmission by aphids tends to occur more frequently near field edges (Masterman et al. 1994). However, most studies show increased numbers of beneficial insects, such as parasitoids, predators, and pollinators, in agrarian landscapes containing diverse edge habitats (Stary & Pike 1999), whereas pest abundance tends to increase in homogenous farm environments (Wilby et al. 2006).

The extent to which habitat defines an insect community can be important from both a biodiversity and applied perspective. Maximizing the complementarity (i.e. dissimilarity) between habitats, or the β -diversity of a landscape, is a key concept in landscape management for biological conservation. At a smaller scale, the community dynamics at ecotones can be important for interpreting how field margins may contribute to diversity within crop-fields. For example, little benefit may be perceived from field margin management in agroecosystems if the ecotone between the crop and the field margin represents a “hard edge” by not allowing beneficial insects to move into the neighboring agricultural field. To date, evidence suggests otherwise. Studies on beneficial epigeal arthropods indicate that field margins may act as refuges and contribute to the abundance of predators within the crop (Kromp and Steinberger 1992, Asteraki et al. 1995). Additionally, some organisms, such as carabids, exhibit cyclic colonization where they may use the open habitat of crop fields for feeding but then retreat to a field margin for overwintering or to escape unfavorable conditions (Dennis et al. 1994, French et al. 2001).

Here, we define the Carabidae community composition and its dynamics across and ag-forest ecotone, consisting of field corn, a mowed grass border, and a forest edge. Within the field corn, we consider and contrast conventional systems and commonly adopted transgenic systems. Using an experimental design of transects across these ecotones, we define the species richness, and the degree of edge effects on community composition. Additionally, the carabid communities found in the cornfields adjacent to the forest are compared to the carabid communities collected in previous studies (see

Chapters 1-3 of this thesis) where plots were embedded in an agricultural landscape, to explore landscape-level influences on carabid diversity in crop fields.

METHODS

This experiment was carried out in an area known as the ridge and valley physiographic province of Pennsylvania, which is characterized by long, limestone or shale, agriculturally-dominated valleys bordered on each side with steep, rocky, sandstone or quartzite forested slopes. Therefore, farms located in the center of the valley are embedded in an agricultural landscape, whereas farms at the edge of the valley share a border with the forested slopes. For this study, three replicate sites of field corn (each separated by $> \frac{1}{2}$ km) adjacent to continuous forest were established at the Russell E. Larson Agricultural Research Center in Rock Springs, PA. Two sites consisted of Andover channery loam soils (AnB and AnC), and the third consisted of Hagerstown silt loam soils (HaA). Each location chosen for the study was planted in field corn the previous year to represent continuous corn management and to establish a baseline community of arthropods. Each site consisted of a paired design with half the acreage planted in transgenic field corn and an adjacent half planted in its near isoline and managed conventionally, so that both genotypes bordered the forested margin. The paired design was used to compare Carabidae diversity measures between transgenic technology and conventional management practices directed toward seed- and root-feeding insect pests.

Field corn treatments. The cornfields at each site varied in size with the smallest being 0.66 ha. Therefore the smallest plot of transgenic or conventional corn

was approximately 0.33 ha. Transgenic field corn was stacked with Roundup-Ready (RR) and YieldGuard Rootworm (YGRW) transgenic technology as well as neonicotinoid / fungicide seed treatments. Specifically, the YGRW field corn (DeKalb DKC-6012, Monsanto Co., St. Louis, MO) expressed Cry3Bb1 proteins for control of root-feeding *Diabrotica* spp., primarily corn rootworm. The seed treatments consisted of a fungicide blend and a neonicotinoid (250 µg clothianidin / seed, Bayer CropScience, Research Triangle Park, NC). The near isoline field corn contained RR technology and the same fungicide blend seed treatment as the transgenic field corn. The isoline seed did not contain a neonicotinoid seed treatment, and therefore received an at-planting soil application of tefluthrin (3.7 kg/ha of Force 3G, Syngenta, Greensboro, NC, 3% AI) for control of corn rootworm and other seed- and root-feeding insects. Both treatments represent common continuous corn insect management practices.

Insect collection and identification. At each site, four transects (two for each field corn cultivar) of pitfall traps were established from the interior of the cornfields, through a narrow grassy field margin (mowed for farmland vehicle use), and into the neighboring forest. Each transect consisted of ten traps: four in the corn, two in the grassy margin, and four in the neighboring forest. Traps in the corn, grass, and forest were spaced 5 m apart except for one distal trap located in the interior of the corn and one distal trap in the interior of the forest (15 m from the penultimate trap in either direction). Pitfall sampling occurred on eight dates at approximately three week intervals spanning late May to late October. Traps were open for 72 hours during each sampling period. All carabids were sorted, pinned and identified to species. Species identification was performed by Robert Davidson (Carnegie Museum of Natural History, Pittsburgh, PA),

David Biddinger (Penn State Fruit Research and Extension Center, Biglerville, PA) and Tim Leslie (Penn State University, University Park, PA) using several taxonomic keys (Downie and Arnett Jr. 1996, Ciegler 2000, Marshall 2006) and voucher specimens from previous studies (Hoheisel 2002, Leslie et al. 2007). Species lists were recorded as baseline information on the carabid fauna found across a maize-forest ecotone. Voucher specimens are housed in the lab of S. J. Fleischer, Department of Entomology at Penn State University, University Park, PA.

Environmental variables. In addition to the nominal treatment variables of management regimes and habitat types, several continuous environmental variables were measured for correlative analyses. All plants were identified and counted at the site of each pitfall trap using a 0.25m² sampling grid to characterize the plant composition of the corn fields, grassy strip, and the forest during the mid-summer. From these data, the number of plant species and the Simpson diversity index were calculated at each trap location. The ground, litter, and canopy cover were also quantified by visual estimation at each trap as a measure of vegetative structural complexity. Plant data were recorded in all habitats in the mid-summer, and once again in the field corn in late summer to represent canopy closure and late-emerging weeds.

Data analysis. The carabid response data was recorded as activity density (# of beetles / trap / 72 hours) for each species. Data analyses were used to compare carabid diversity (species richness and evenness), complementarity (similarity indices) and community structure (ordination) between treatments, habitats, and position along the ecotone. At a larger spatial scale, we also compared the carabid community found in corn adjacent to forest (referred to hereafter as, “forest-near corn”) to those found from

the previous studies (see Chapters 1-3 of this thesis) where fields of similar corn cultivars were surrounded by other agricultural fields and not adjacent to forest (referred to hereafter as, “forest-far corn”).

Carabidae species richness and evenness (dominance distribution) were compared between transgenic technology and conventional management treatments and between habitat types (corn, grass, and forest). Additionally, carabid species accumulation and evenness in the forest-near corn was compared to that found in forest-far corn from other studies. Species richness was examined using rarefaction curves generated in EstimateS (Colwell 2005). Rarefaction allows for comparison of species accumulation curves between treatments or habitats. Through random re-sampling of the data, rarefaction produces curves representing a statistical expectation of species accumulation with increasing sampling effort (i.e., number of samples, or number of individuals collected). The permutational procedure also generates confidence intervals allowing for statistical comparison between treatments. Using interpolation, species richness can then be compared between treatments at a uniform sampling effort (Gotelli and Colwell 2001). Individual-based rarefaction curves were used for all comparisons of carabid species accumulation. Evenness of the carabid community was examined by comparing dominance distributions of the carabids. Dominance distribution was visualized by plotting the relative abundance of each species in decreasing order, and the dominant members of the carabid community were compared between treatments and habitats.

To examine the influence of the adjacent habitat on the carabid diversity within the cornfields, we first measured similarity using the classic Sørensen and Jaccard indices, which measures similarity based on the number of shared species and number of

unique species in pairwise comparisons of communities (Magurran 2004). Also included in the similarity analyses are the abundance-based Sørensen and Jaccard indices, which use not only the number of shared or unique species, but also consider abundance (instead of presence/absence) in the calculation of the index (Chao et al. 2005). Similarity was compared between the carabid communities in each of the three different habitats. Additionally, similarity indices were calculated for the carabid communities found in forest-near corn and the forest-far corn.

We used ordination to identify species groupings within the carabid community based on their association with explanatory variables measured or recorded at each trap location. Response data were Hellinger transformed prior to analysis (Legendre and Gallagher 2001). Explanatory variables described the various habitats (i.e., corn, grass, and forest), and the vegetation structure (i.e., % canopy cover, % ground cover, % litter cover) and plant diversity (i.e., # plant species, Simpson diversity index value) found within them. The species-environment associations were visualized in bi-plots from a redundancy analysis (RDA) performed in CANOCO 4.5 (ter Braak and Šmilauer 2002). A forward selection procedure using Monte Carlo permutations was used to identify the most influential environmental variables on carabid community composition.

We created a novel adaptation of principal response curve analysis (PRC) to investigate carabid community dynamics across the transect extending from interior forest, through a grassy strip, to interior corn. PRC is a form of RDA that is analogous to a multivariate repeated measures because it measures community dynamics at multiple time points. In a PRC, the dynamics of treatment communities are measured in relation to the dynamics of a control community over time. PRC is especially useful in

community-level ecotoxicological before-after / control-impact (BACI) experiments to see if an external input results in the treated communit(ies) deviating from the control community, and when this deviation occurs in relation to the timing of the input (ter Braak and Šmilauer 2002). For this study, we pooled the data from all time points and used PRC to examine community dynamics over a spatial, as opposed to a temporal, gradient. We identified the control community as the carabids collected in the trap located in the interior forest, as this habitat is the furthest from the corn field and is theoretically the least disturbed environment. Carabid communities collected at each trap location along the transect were then compared to the forest interior community. The dissimilarity between the control community and the community at each trap location was enumerated with canonical coefficients that were plotted on a distance-based gradient. On the diagram, the control community is represented as a horizontal line set to 0.0, and the plotted canonical coefficients represent the spatial trajectory of community differentiation. Since each trap location was associated with a certain habitat, we then overlaid habitat delineations on the PRC diagram to see how communities responded at specific ecotones (i.e., forest-grass and grass-corn).

In addition to the principal response trajectory, the PRC procedure provides taxa weights for each species in the community. The taxa weights represent how closely each species follows the principal response. A positive taxon weight indicates 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 strong relationship. Taxon weights between -0.5 and 0.5 are generally

considered insignificant, and species falling between these values are not shown in the diagram.

Species-level taxonomic resolution allowed us to also investigate population-level trends at the forest edge. For each of the abundant carabid species, we plotted the distribution of trap captures at each location on the transect. We compared these spatial distributions to the patterns found in the RDA and PRC, and identified which species were habitat specialists or generalists. We also identified forest species that may contribute to farmland diversity by moving across the ecotones.

RESULTS

Species richness. The forest carabid community exhibited significantly higher species richness than the carabid community in corn (Fig. 1) based on non-overlapping confidence intervals. The grass community fell in between forest and corn but was not significantly different from either. At a rarefied level of 300 individuals, the forest community had accumulated an average of 50 species, compared to 40 species in grass, and 29 species in corn. Based on the length of the rarefaction curves, carabid activity-density (i.e., # of individuals collected) was two to three times greater in corn than in grass and forest (Fig. 1). Species richness was not significantly different between management regimes within the corn, however species richness estimates were higher in the corn utilizing transgenic technology (Fig. 2). Activity-density, indicated by the length of the rarefaction curve, was higher in the conventionally managed field corn.

At the landscape level, carabid species richness estimates varied between the forest-near corn and the forest-far corn. In most instances, richness estimates in the

forest-near corn were equal to or higher than the forest-far corn. At a rarefied value of 300 individuals, forest-near corn (Fig. 1) had accumulated a mean of 30 species, whereas forest-far corn accumulated anywhere from 14 to 32 species (see: Figure 4 in Chapter 1, and Figure 1 in Chapter 3).

Evenness. The carabid community in corn and grass exhibited a highly skewed dominance structure with a single species, *Harpalus pensylvanicus*, representing 59% and 48% of the total abundance in each habitat, respectively (Fig. 3). The remaining species in field corn all represented < 10% of the total abundance, while in grass only one other species, *Poecilus lucublandus*, occurred at >10% of the total abundance. Despite the similar shape in dominance structure, the dominant species varied between the corn and grass, as only four species were among the ten most abundant in both habitats (*H. pensylvanicus*, *Poecilus lucublandus*, *Pterostichus stygicus*, and *Cyclotrachelus furtivus*). The forest community exhibited a vastly different, and more even, community structure (Fig. 3). No species represented >15% of the total abundance. Of the ten most abundant species in the forest, four were among the most abundant in grass (*P. stygicus*, *P. lucublandus*, *Pterostichus mutus*, and *Calathus gregarius*) and three among the most abundant in corn (*P. stygicus*, *P. lucublandus*, and *Patrobus longicornis*). Differences in dominance structure between insect pest management tactics within the corn were minimal (Fig. 4). Both treatment communities were dominated by three species, *H. pensylvanicus*, *P. lucublandus*, and *C. tricolor tricolor*), and eight of the ten most abundant species in each treatment community were shared.

The distribution of dominance in forest-near corn was similar (i.e. highly skewed) to the distribution in forest-far corn, however the species composition varied (compare

Fig. 4 in this chapter with Fig. 5 in Chapter 1 and with Fig. 1 in Chapter 2). While *H. pensylvanicus* was found in high numbers in all areas, the second most abundant forest-near carabid, *P. lucublandus*, tended to be found in lower percentages in the forest-far corn. In the forest-far corn, *Poecilus chalcites*, *S. quadriceps*, *Bembidion quadrimaculatum oppositum*, and *P. melanarius* all tended to be among the most dominant carabids, yet were not found in high numbers in the forest-near corn. In fact, only two (*H. pensylvanicus* and *P. melanarius*) of these five forest-far corn species were among the top ten ranked species in forest-near corn. Other abundant species in forest-near corn, such as *P. stygicus*, *D. elongatus*, *C. furtivus*, and *A. littoralis* were either found in very low numbers or not found at all in forest-far corn.

Similarity. A comparison of the three edge habitats, revealed carabid communities in the corn and grass resulted in the highest similarity indices, grass and forest communities were the second most similar, and corn and forest communities were the least similar (Table 2). When comparing similarity between forest-near corn and forest-far corn, the community composition differences noted in the dominance distribution analyses were further supported by the similarity indices. In the pairwise comparisons, the forest-near corn community was consistently less similar to the three forest-far corn communities than the forest-far communities were to one another (Table 2). Additionally, the carabid communities in forest-near corn and the adjacent grassy habitat were more similar than the forest-near corn community and the forest-far corn communities, indicating substantial species exchange between adjacent habitats. These similarity trends held true across both the classic and abundance-based Jaccard and Sørensen indices.

Community structure. RDA identified significant axes, or gradients, of variation in the carabid community collected in this study (Fig. 5; Axis 1: $F=15.27$, $P=0.002$; Axis 2: $F=3.72$, $P=0.002$). The nominal explanatory variables defining habitat type (corn, grass, and forest) are depicted on the RDA bi-plot (Fig. 5) with diamonds, while the continuous variables associated with plant diversity and structure (Litter cover, ground cover, canopy cover, species richness, and Simpson Diversity Index) are shown as bolded vectors. Carabid species, the response variables, are shown as the lighter vectors and the names are abbreviated (refer to species list in Table 2 for full name). Figure 5 can be used for visualization of the results reported below. The primary, horizontal, axis was associated with field corn ($F=17.65$, $P=0.002$) communities separating from corn and grass communities, and explained 37% of the constrained carabid species variation. The secondary, vertical, axis was associated with % ground cover ($F=2.99$, $P=0.002$) differentiating from % canopy cover. Several of the plant diversity and structure variables were correlated with the habitat variables; for example, increasing litter cover was highly associated with the forest, and increasing ground cover was highly associated with the grass. Canopy cover was associated with both forest and corn, and negatively associated with grass. Increasing plant diversity as measured by number of species (“Richness”) and the Simpson Diversity Index (“Index”) were found to be associated with forest and grass, and negatively associated with corn.

Carabidae species could be grouped based on their association with a habitat or plant diversity and structure. *P. longicornis*, *C. emarginatus*, *S. impunctatus*, *P. mutus*, *S. stenostomus*, and *P. stygicus* comprised the forest grouping, with its higher litter, canopy, and plant diversity values. *C. furtivus*, *C. seguttata*, *P. melanarius*, and *C. tricolor*

tricolor were associated with the corn along this forest edge. *H. pensylvanicus* and *P. lucublandus* were primarily found in both corn and grass, while *C. gregarius* was more closely associated with the second axis, or ground cover (which is highly correlated with the grass).

The species with the shortest vectors, *D. elongatus*, *C. furtivus*, *P. lucublandus*, and *P. longicornis*, may represent habitat generalists, as the short vectors represent relatively weak correlations with the explanatory variables. Even other species that showed a strong correlation with a given habitat, such as *P. stygicus* and its association with forest, were found among the various habitats. In fact, despite its strong association with forest, *P. stygicus* was still among the most abundant species found in corn (Fig. 3). Therefore, strong species-habitat associations do not necessarily indicate that ecotones represent a hard edge to these species.

Community shift across transect. The spatial trajectory of the carabid community across the transect significantly deviated from the baseline interior forest carabid community ($F=45.6$, $P=0.002$) as shown by the PRC diagram (Fig. 6). The community trajectory across the transect exhibited a logistic shape in dissimilarity distribution, with the greatest shift, or most rapid deviation from the interior forest community, occurring in the grass habitat between the forest and corn. The first four trap sites situated in the forest showed a gradual linear decline in an order that was consistent with their proximity to the edge. At the ecotone of forest and grass a distinct community shift occurred, followed by another distinct drop to the next trap in grass adjacent to the corn. At the ecotone of grass and corn, there was no abrupt change in community structure. Within the corn, PRC scores increased slightly, again in an order that was

consistent with their proximity to the edge, and then stabilized along the transect toward interior corn. Therefore, the carabid community collected in traps immediately surrounding the corn-grass ecotone, were the most dissimilar to the interior forest community based on PRC scores.

Species weights were used to identify the degree at which different species followed the principal response. Species with a significant positive species weight (>0.5) were those that strongly followed the principal response, whereas those with a significant negative species weight (<-0.5) responded in the opposite fashion. The carabids with positive species weights, *S. impunctatus*, *S. stenostomus*, *P. mutus*, and *C. emarginatus*, were those highly associated with the forest (Fig. 5) and exhibited the least overlap with the other habitats (Fig. 7). Therefore, they would follow the pattern seen in the principal response, since they would decrease in abundance as distance from the interior forest increases. Conversely, the carabids with a negative species weight, *P. lucublandus* and *H. pensylvanicus*, were highly associated with grass and corn, and increased in number as distance from interior forest increased, thus acting in an opposite manner of the community trajectory in the PRC diagram.

Population distributions. The spatial distribution of activity-densities for each species at the forest edge (Fig. 7-9) supported the habitat groupings identified in the RDA bi-plot (Fig. 5), however the extent of movement of each species into other habitats was more clearly displayed. Of the 14 most abundant species, none were confined to a single habitat, and only three were not found in all three habitats. Four species (Fig. 7: *C. tricolor tricolor*, *P. melanarius*, *C. sexguttata*, *H. pensylvanicus*) were identified as corn species, five species (Fig. 8: *C. emarginatus*, *P. stygicus*, *P. mutus*, *S. stenostomus*, *S.*

impunctatus) were identified as forest species, and five species (Fig. 9: *P. longicornis*, *C. gregarius*, *C. furtivus*, *D. elongatus*, *P. lucublandus*) were identified as habitat generalists. The corn species displayed considerable movement into the grass, and all, except for *P. melanarius*, had a few transient individuals in the forest (Fig. 7). Of the forest species, *C. emarginatus*, *P. stygicus*, and *P. mutus* had distributions that extended into the corn, whereas *S. stenostomus* and *S. impunctatus* were confined to the forest and grass (Fig. 8). Those species identified as habitat generalists in the RDA (species with short vectors in Fig. 5) were found to have distributions that were relatively even across the habitats, except *C. furtivus*, which was found more often in grass and corn (Fig. 9), indicated by the direction of its vector in Figure 5. *C. gregarius*, which was identified as a grass species based on the RDA bi-plot (Fig. 5), was grouped with the habitat generalists, as the grass was situated between the corn and forest and a noticeable number of *C. gregarius* were found in both the corn and forest, at all trap locations (Fig. 9).

DISCUSSION

The evolutionary success of Carabidae is readily apparent in their species richness (> 40,000 species worldwide) and the diversity of habitats to which they have adapted, including frequently disturbed habitats such as agricultural fields. Carabidae have the propensity to be useful bioindicators of farm management practices because they are abundant in agricultural settings, relatively easy to collect, species identification is possible, and their composition and abundance can be influenced by management (Duelli et al. 1999). For the most part, carabids are considered beneficial predators of invertebrate pests and weed seeds in agricultural fields (Toft and Bilde 2002). Although

many carabids can be closely linked to specific habitats, other species may rely on multiple habitats for reproduction, survival, or feeding (Dennis et al. 1994, French et al. 2001). Therefore, field margins may represent a key component of agroecosystems for developing more abundant and species-rich carabid assemblages within crop fields, and can be an area of study that encompasses the fields of sustainable agriculture, conservation biological control, and landscape ecology.

This study supports the notion that landscape-scale factors are influential in defining local insect diversity (e.g., Purtauf et al. 2005). Based on shared-species similarity indices, carabid communities collected among forest-far cornfields were consistently more similar than when compared to the forest-near carabid community (Table 2). While the shape of the dominance distribution was similar in both areas (Fig. 4, and Fig. 5 in Chapter 1 and Fig. 1 in Chapter 2), the dissimilarity between communities in forest-near corn and forest-far corn was driven by shifts in the relative abundance of shared species and the acquisition of new species that favor the forest edge habitat. For example, *P. lucublandus* and *P. chalcites* seem to shift roles in dominance based on proximity to forest; *P. lucublandus* was found in higher abundance near the forest edge, whereas *P. chalcites* was a dominant member of forest-far corn. Indeed the distribution of *P. lucublandus* across the transect shows a strong presence in all three habitats (Fig. 9), and suggests that this species benefits greatly from the edge habitat. Other species, such as *P. stygicus*, *C. emarginatus*, *D. elongatus*, *C. furtivus*, *P. longicornis*, were relatively abundant in the forest-near corn, but were rarely found (i.e., transient catches), or not found at all, in the forest-far corn. As with *P. lucublandus*, these species clearly benefit

from the edge habitat as generalists (Fig. 9), or they behave as forest species with sufficient movement into the corn (Fig. 8).

Despite the apparent influence of the forest edge on carabid community composition in the corn, it is yet unclear whether this represents simply a community shift (in comparison to forest-far corn), or an actual increase in diversity within agroecosystems at the forest edge location. Comparisons of species richness suggest equal or higher diversity in the forest-edge corn (compare Fig. 1 in this chapter to Fig. 4 in Chapter 1 and Fig. 1 in Chapter 3), however a replicated experiment during the same year would be needed for a reliable statistical comparison. Such a study would be warranted, as there is increasing evidence of a positive relationship between biodiversity and ecosystem function (Cardinale et al. 2006), and when considering multiple ecosystem functions (as is often the case in agriculture), even higher levels of biodiversity may be needed (Hector and Bagchi 2007). These positive relationships are found to be asymptotic due to functional redundancy, however this redundancy can act as a “buffer”, adding stability to an ecosystem through a more diverse species pool (McCann 2000, Tilman et al. 2006).

At the local scale, community shifts at the interface of the field corn and the field margin were investigated. Results consistently identified a strong separation between the forest carabid community and the grass and corn carabid community. An RDA identified habitat type as the dominant factor defining insect community composition, where the forest differentiated strongly from the corn and grass (Fig. 5), and grass and corn differentiated to a lesser extent along the vertical axis of the bi-plot (Fig. 5). Similarity indices also indicated that grass and corn were more similar than grass-forest

or corn-forest (Table 2). This pattern was re-enforced by the PRC where a distinct shift in the carabid community was seen at the forest-grass ecotone (Fig. 6), but not at the grass-corn ecotone (Fig. 6). However, it should be noted that the principal response was based on dissimilarity with interior forest, which we defined as our baseline, so the carabid communities in grass and corn could have been widely divergent, yet equally dissimilar from interior forest. This emphasizes the importance of using multiple approaches for examining community patterns. In this case, the RDA and similarity indices were complementary to the PRC, by identifying differences between the carabid communities in corn and grass that was not possible in the PRC. For example, according to the PRC, the carabid community found near the grass-corn ecotone was the most dissimilar to the interior forest community, whereas toward the interior corn PRC scores increased slightly indicating a more similar community to the interior forest, despite being more spatially distinct. This can be at least partially explained by looking at the species-level distributions; *H. pensylvanicus*, the most abundant carabid, was rarely found in the forest (Fig. 7) and was strongly correlated to the principal response (Fig. 6). The abundance distribution of *H. pensylvanicus* along the transect clearly shows a preference for the grass-corn ecotone (Fig. 7), which may be related to its functional role as a weed seed predator; weed invasion at the field edge may have provided a more substantial food source at this location. Although *H. pensylvanicus* was also abundant in the interior corn, it was proportionally lower than the field edge, which can explain the increased PRC score in the interior corn.

In addition to the segregation of species among habitats and their defining plant characteristics (Fig. 5), it is important to consider the sharing of species among habitats.

Species-level identification allows for population-level analyses that can complement investigations into community-level trends. For example, from the community analyses we might conclude that the forest-grass ecotone acts as a hard edge, due to the distinct community shift at the ecotone in the PRC, and the strong gradient between forest and the other habitats on the RDA bi-plot. However, at the landscape-level we also detected noticeable differences in community composition between carabid communities in forest-near corn and forest-far corn. These results were partially explained by looking at the distribution of each species separately along the transect. While species may be strongly associated with a certain habitat (Fig. 5), we rarely found a species that was not captured within multiple habitats (Figs. 7-9), which helps explain our differences in landscape-scale patterns. Bedford and Usher (1994) noted this “sharing” of species between agricultural and forested habitats, and we also documented forest species in the corn habitat (Fig. 8) and conversely, corn species in the forest (Fig. 7).

Although there were prominent differences in carabid communities between habitat types, no significant differences in carabid communities were detected between insect control practices within the corn. Dominant community members and their distribution of abundances were similar between both the transgenic and conventionally managed corn. Carabid communities had higher species richness measurements in the transgenic corn (Fig. 2), however this was not significant based on confidence intervals. Any direct effect of the transgenic corn on carabids would be most likely due to the neonicotinoid seed treatment and not the Cry3Bb proteins (Mullin et al. 2006). The seed treatment represented a different insecticide delivery system than the conventional field corn, which received an at-planting soil pyrethroid application, possibly affecting carabid

communities differentially. However, when compared to a control, carabid communities exhibited a similar response in both transgenic corn with a neonicotinoid seed treatment and conventionally managed field corn receiving soil pyrethroid at planting (see Chapters 3 and 4). Another study in field corn found effects from both insect control methods, however more often from soil insecticides and not seed treatments (Bhatti et al. 2005). In a diversified vegetable farm setting, crop species and crop rotation influenced insect communities more than any difference in common pest management inputs (Leslie et al. 2007).

Despite the abundance of carabids found in the corn, diversity was still higher in the forest (Fig. 1), and grass to a lesser extent, where plant diversity measurements were also higher (Fig. 5). It is difficult to determine whether this bottom up influence is driven by the taxonomic diversity of plants, a disturbance gradient, or the increase in structural complexity (i.e., more microhabitats) that they represent, because all are highly correlated; it is likely that all play some role. There is increasing evidence that vegetation structural diversity plays a larger role in supporting carabid diversity, than does plant taxonomic diversity (Brose 2003). In our findings, plant species richness and diversity (based on Simpson diversity index) were highest in the grass and corn. However, vegetation structure was different between the two habitats, as grass had the highest % ground cover, and forest was highest in % litter and canopy cover. Since carabid species richness estimates were highest in the forest, this suggests that vegetation structure or level of disturbance (i.e., grass was mowed) may play an important role in defining carabid diversity. In the corn, where carabid species richness was the lowest, the vegetation structure associated with the carabids ground-dwelling habitats (i.e., % litter

and % ground cover) were also the lowest. However, activity-density of carabids was high, as the open environment was conducive to movement. In either case, these findings highlight the importance of species-rich and structurally diverse plant communities in field margins for supporting carabid biodiversity and β -diversity in agricultural landscapes.

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Table 1. Carabidae collected in Rock Springs, PA along a transect spanning field corn, grass, and forest.

<i>Agonum cupripenne</i>	<i>Bembidion rapidum</i>	<i>Platynus decentis</i>
<i>Agonum ferreum</i>	<i>Bradycellus lugubris</i>	<i>Poecilus chalcites</i>
<i>Agonum melanarium</i>	<i>Bradycellus tantillus</i>	<i>Poecilus lucublandus</i>
<i>Agonum muelleri</i>	<i>Calathus gregarius</i>	<i>Pseudamara arenaria</i>
<i>Agonum palustre</i>	<i>Chlaenius emarginatus</i>	<i>Pterostichus adoxus</i>
<i>Agonum punctiforme</i>	<i>Chlaenius tricolor tricolor</i>	<i>Pterostichus caudicalis</i>
<i>Agonum retractum</i>	<i>Cicindela punctulata punctulata</i>	<i>Pterostichus commutabilis</i>
<i>Amara aenea</i>	<i>Cicindela sexguttata</i>	<i>Pterostichus coracinus</i>
<i>Amara apicaria</i>	<i>Clivina impressifrons</i>	<i>Pterostichus luctuosus</i>
<i>Amara cupreolata</i>	<i>Cyclotrachelus furtivus</i>	<i>Pterostichus melanarius</i>
<i>Amara exrata</i>	<i>Cymindis platicollis</i>	<i>Pterostichus mutus</i>
<i>Amara familiaris</i>	<i>Cymindis cribricollis</i>	<i>Pterostichus pensylvanicus</i>
<i>Amara impuncticollis</i>	<i>Dicaelus elongatus</i>	<i>Pterostichus rostratus</i>
<i>Amara littoralis</i>	<i>Dicaelus politus</i>	<i>Pterostichus stygicus</i>
<i>Amara musculus</i>	<i>Dyschirius globulosus</i>	<i>Pterostichus tristis</i>
<i>Amara ovata</i>	<i>Harpalus compar</i>	<i>Scarites quadriceps</i>
<i>Amara rubrica</i>	<i>Harpalus erythropus</i>	<i>Scarites subterraneus</i>
<i>Amphasia interstitialis</i>	<i>Harpalus herbivagus</i>	<i>Sphaeroderus stenostomus</i>
<i>Anisodactylus harrisii</i>	<i>Harpalus longicollis</i>	<i>Stenolophus conjunctus</i>
<i>Anisodactylus melanopus</i>	<i>Harpalus pensylvanicus</i>	<i>Stenolophus rotundatus</i>
<i>Anisodactylus nigerrimus</i>	<i>Harpalus protractus</i>	<i>Stenolophus rotundicollis</i>
<i>Anisodactylus nigrita</i>	<i>Harpalus rubripes</i>	<i>Syntomus americanus</i>
<i>Anisodactylus rusticus</i>	<i>Harpalus somnulentus</i>	<i>Synuchus impunctatus</i>
<i>Anisodactylus sanctaerucis</i>	<i>Notiobia nitidipennis</i>	<i>Trichotichnus fulgens</i>
<i>Apenes lucidulus</i>	<i>Notiophilus aeneus</i>	<i>Trichotichnus autumnalis</i>
<i>Badister notatus</i>	<i>Olisthopus parmatus</i>	<i>Trichotichnus dichrous</i>
<i>Bembidion affine</i>	<i>Oodes amaroides</i>	<i>Trichotichnus vulpeculus</i>
<i>Bembidion mimus</i>	<i>Patrobus longicornis</i>	<i>Xestonotus lugubris</i>
<i>Bembidion quad. opp.</i>		

Table 2. Pairwise comparisons of Carabidae communities sampled from different cornfield locations and non-crop habitats using multiple similarity indices. A higher value indicates greater similarity. Corn (FE) = field corn adjacent to a forest edge; Sweet Corn = sweet corn from a diversified vegetable farm embedded in a primarily agricultural landscape; Corn (2003) and Corn (2004) = field corn embedded in a primarily agricultural landscape in 2003 and 2004, respectively.

Scale	Habitat Comparison	Shared Species	Similarity Index			
			Jaccard Classic abundance-based	Jaccard abundance-based	Sørensen Classic	Sørensen abundance-based
Local (forest edge)	Corn (FE) vs. Grass	30	0.508	0.883	0.674	0.938
	Corn (FE) vs. Forest	21	0.284	0.586	0.442	0.739
	Grass vs. Forest	23	0.307	0.683	0.469	0.811
Landscape (near vs. far)	Corn (FE) vs. Sweet Corn	21	0.375	0.833	0.545	0.909
	Corn (FE) vs. Corn (2003)	24	0.393	0.722	0.565	0.839
	Corn (FE) vs. Corn (2004)	18	0.305	0.772	0.468	0.871
Local (far)	Sweet Corn vs. Corn (2003)	30	0.652	0.870	0.789	0.930
	Sweet Corn vs. Corn (2004)	22	0.478	0.887	0.647	0.940
	Corn (2003) vs. Corn (2004)	29	0.617	0.892	0.763	0.943

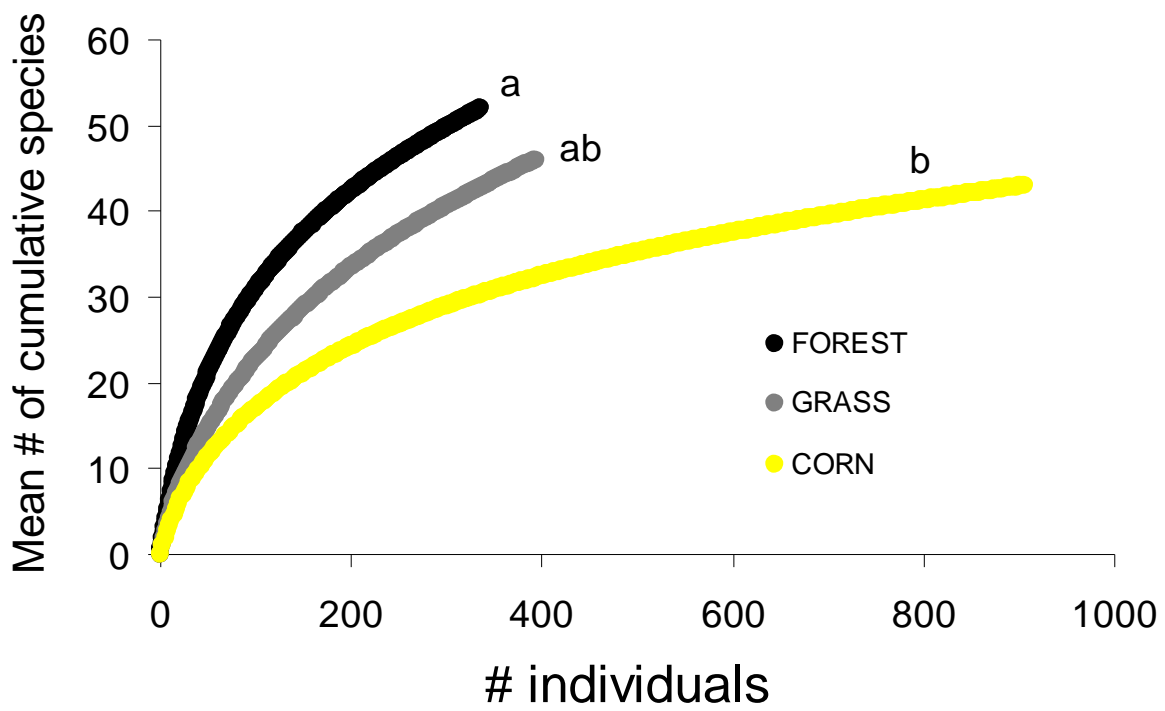


Figure 1. Individual-based rarefaction curves depicting Carabidae species accumulation in forest, grass, and field corn.

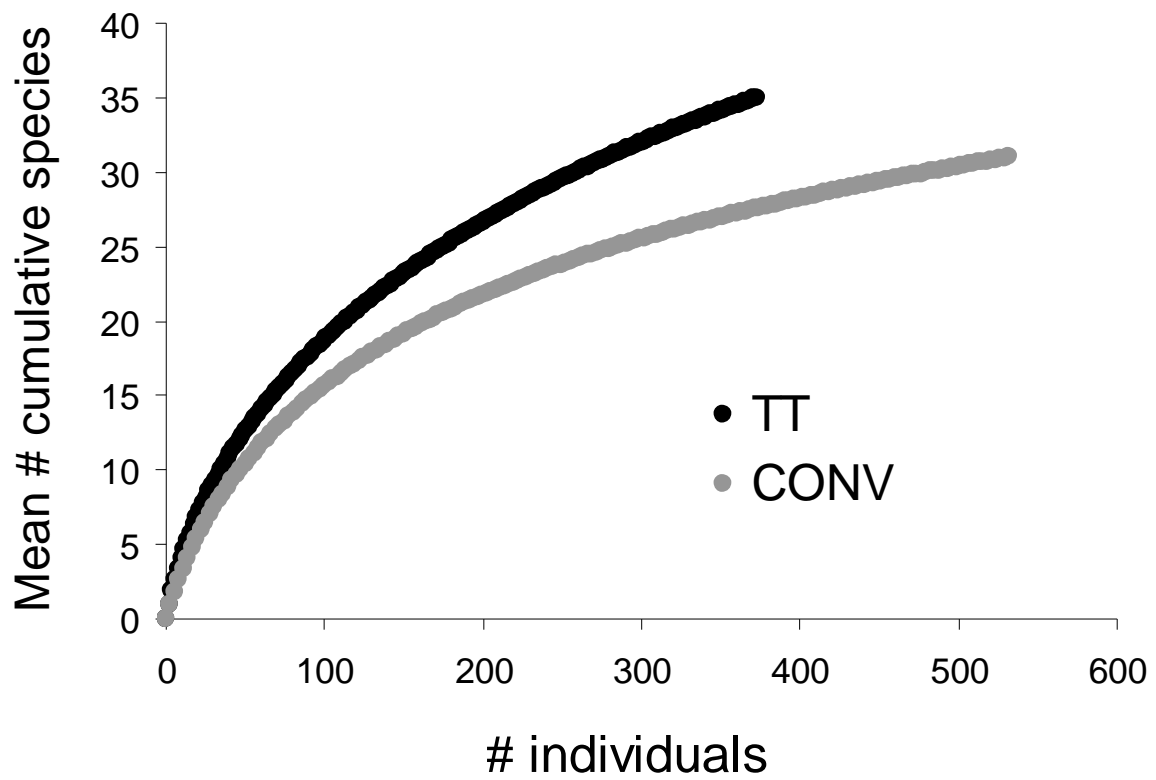


Figure 2. Individual-based rarefaction curves depicting Carabidae species accumulation in no-till field corn managed with transgenic technology (TT) or conventional insecticides (CONV).

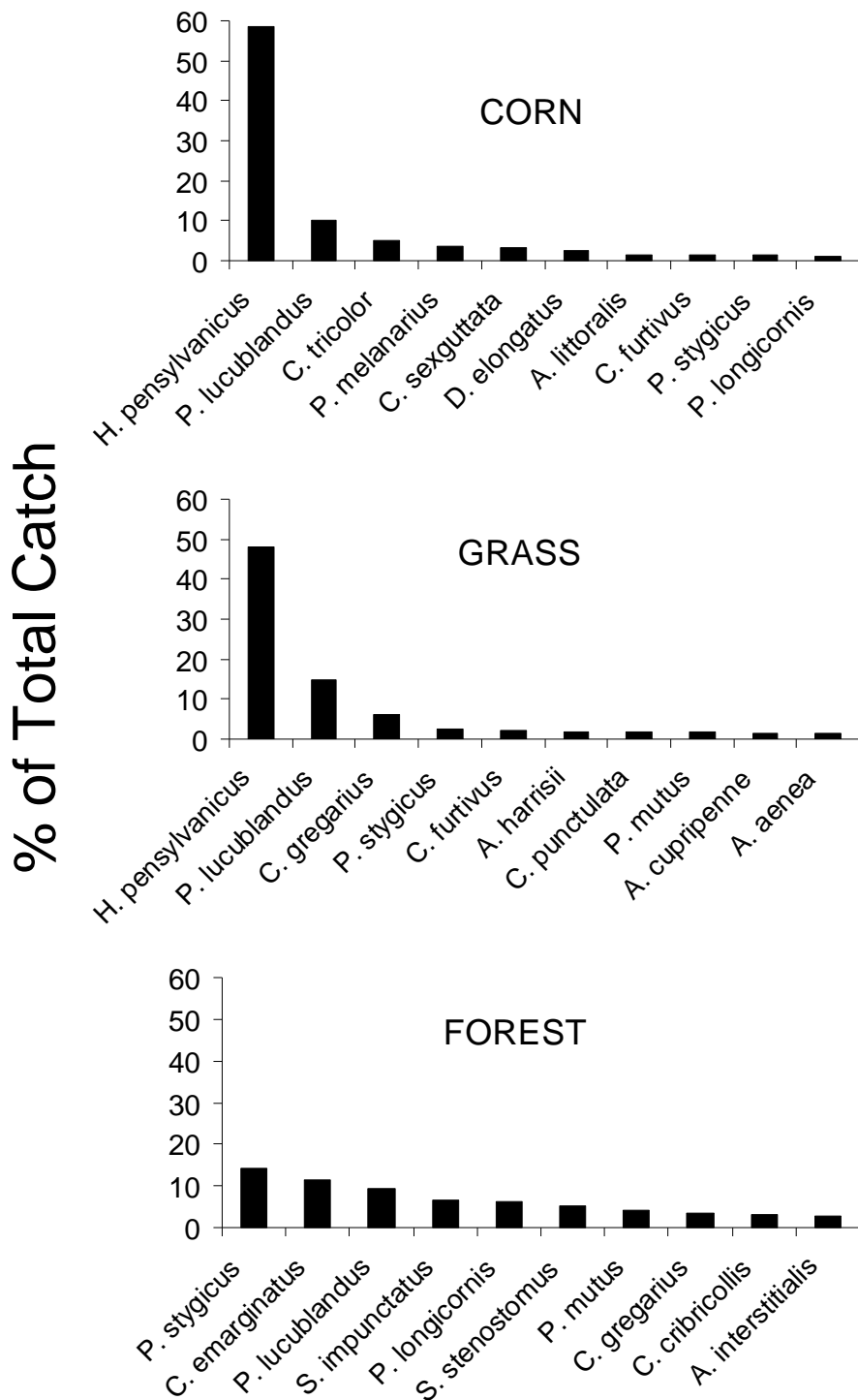


Figure 3. Dominance distribution of most abundant carabids in collected in field corn, grass, and forest.

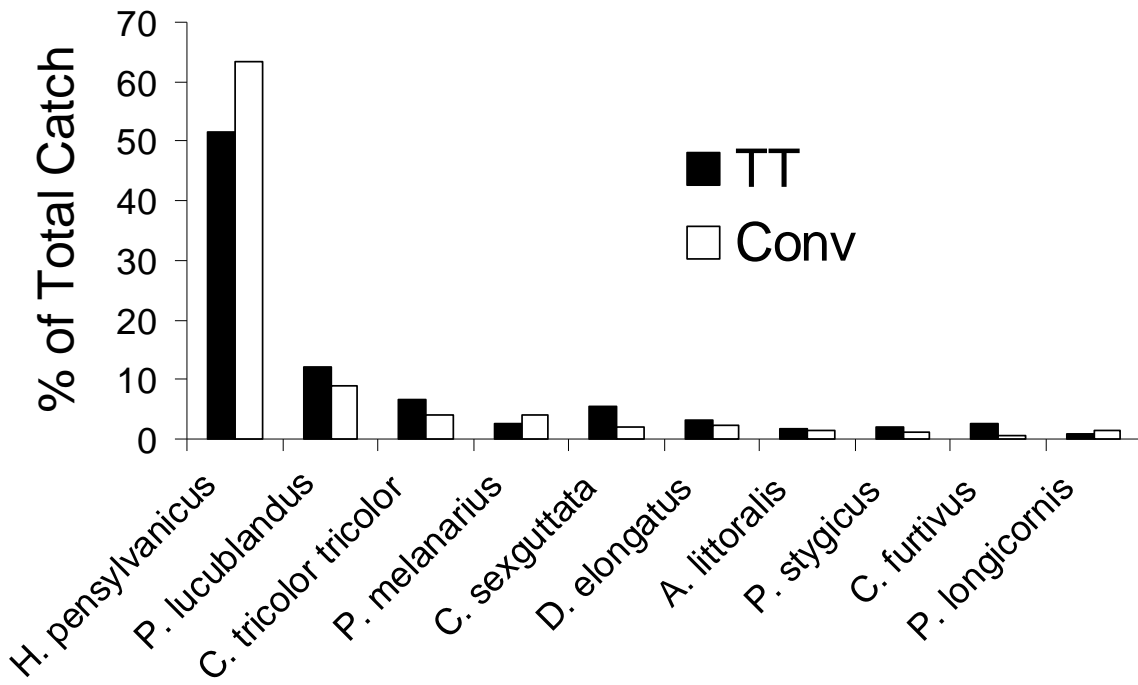


Figure 4. Dominance distribution of most abundant carabids collected in no-till field corn managed with transgenic technology (TT) or conventional insecticides (CONV).

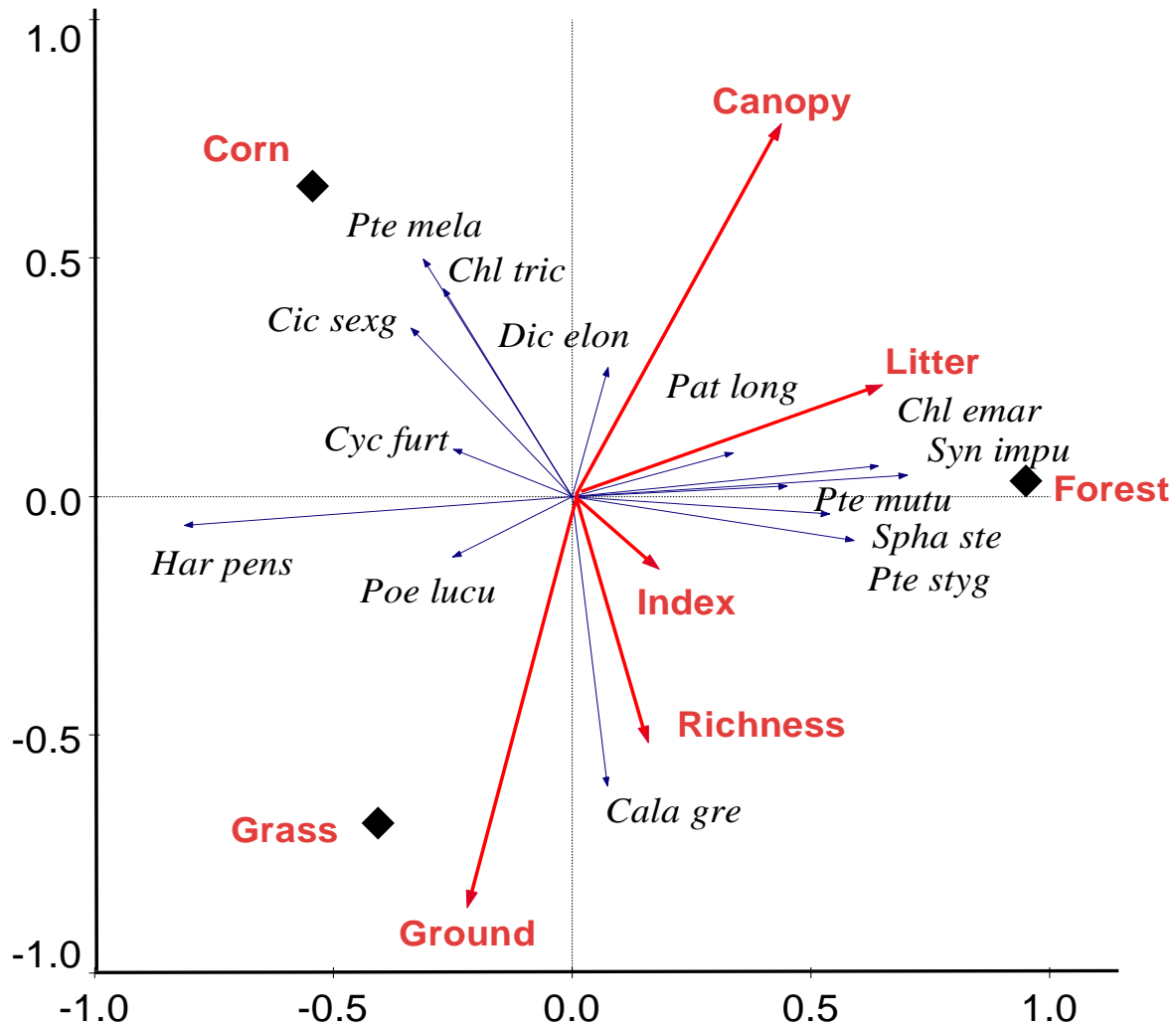


Figure 5. Redundancy analysis (RDA) bi-plot depicting associations between carabids and environmental (i.e., explanatory) variables. Community gradients (axes) are constrained by the explanatory variables. Nominal explanatory variables, shown as diamonds, are habitat types (i.e., forest, grass, corn). Continuous explanatory variables, shown as bolded vectors, are associated with plant diversity (i.e., plant richness or “Richness”, and Simpson index of plant diversity or “Index”) and vegetation structure (i.e., % Litter, % Ground, and % Canopy cover). Carabid species, represented by lighter vectors, are abbreviated with the first three letters of their genus and first four letters of the species epithet. Refer to Table 1 for complete species names.

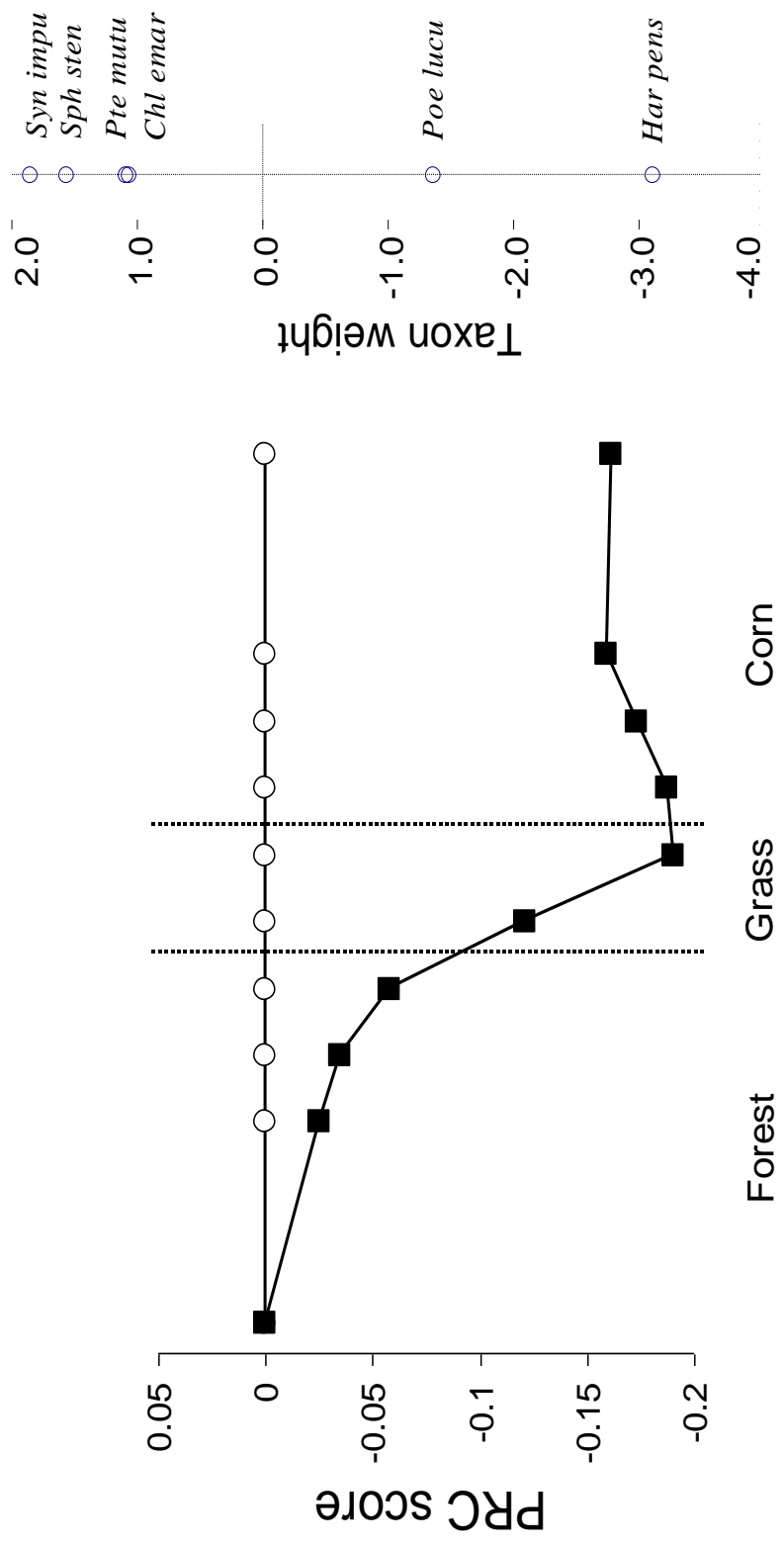


Figure 6. Principal response curve (PRC) and taxon weights indicating shifts in the carabid community structure across a ten-trap transect extending from the interior of a forest (n=4), through a grassy margin (n=2), and into the interior of a neighboring cornfield (n=4). Traps were 5m apart except for the distal traps, which were situated 15m from the adjacent trap. The shifts in carabid community composition represented by the PRC (black squares) are shown in relation to the interior forest carabid community (shown as open circles on the horizontal 0.0 line). Species with positive species weights (> 0.5) follow the principal response, whereas species with negative species weights (< -0.5) responded in the opposite fashion. Vertical lines indicate where two habitats intersect.

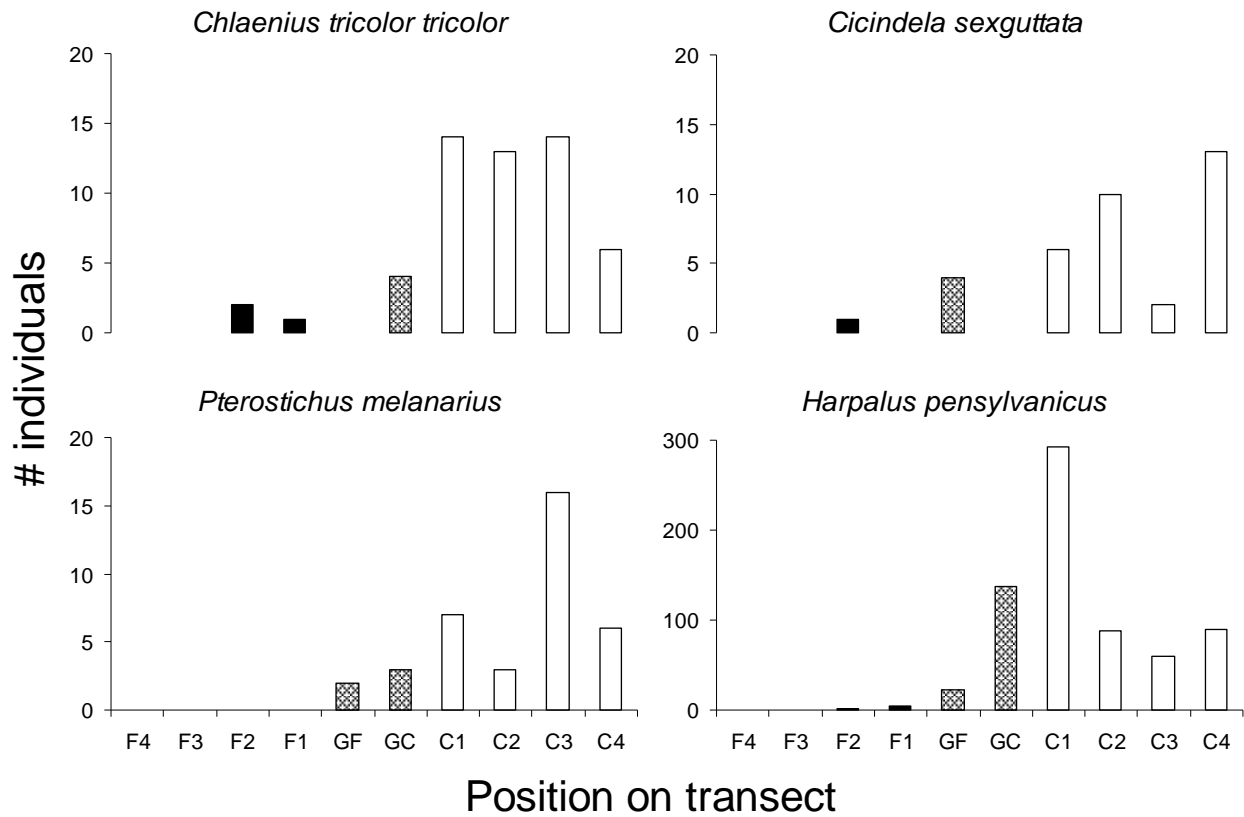


Figure 7. Distribution of four carabid species along a transect extending from forest (F4-F1), through a mowed grassy margin (GF and GC), and into corn (C1-C4). Traps were 5m apart except for the distal traps (F4 and C4), which were situated 15m from the adjacent trap. Carabid species in this figure were found to be highly associated with the field corn habitat.

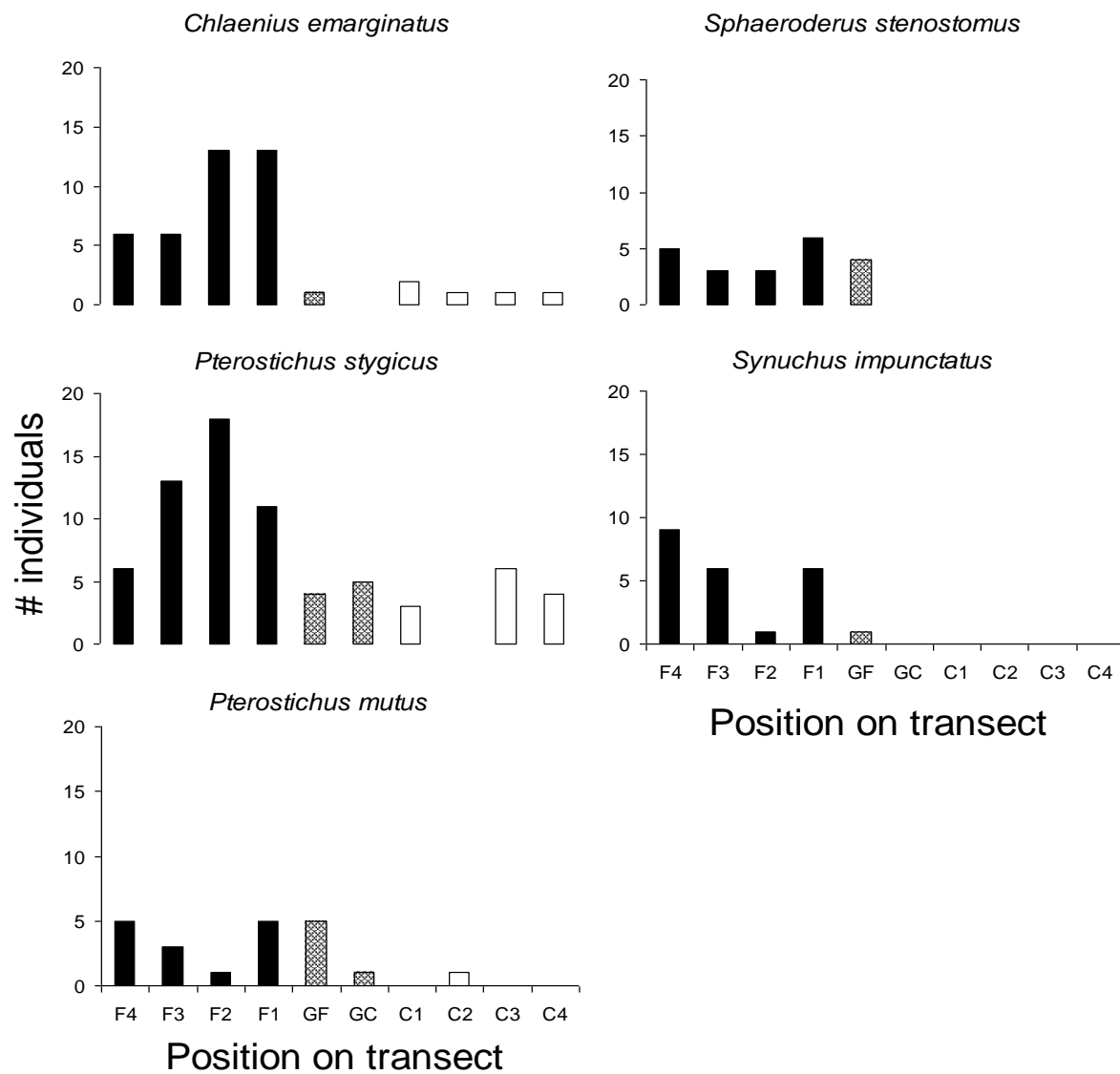


Figure 8. Distribution of five carabid species along a transect extending from forest (F4-F1), through a mowed grassy margin (GF and GC), and into corn (C1-C4). Traps were 5m apart except for the distal traps (F4 and C4), which were situated 15m from the adjacent trap. Carabid species in this figure were found to be highly associated with the forested habitat.

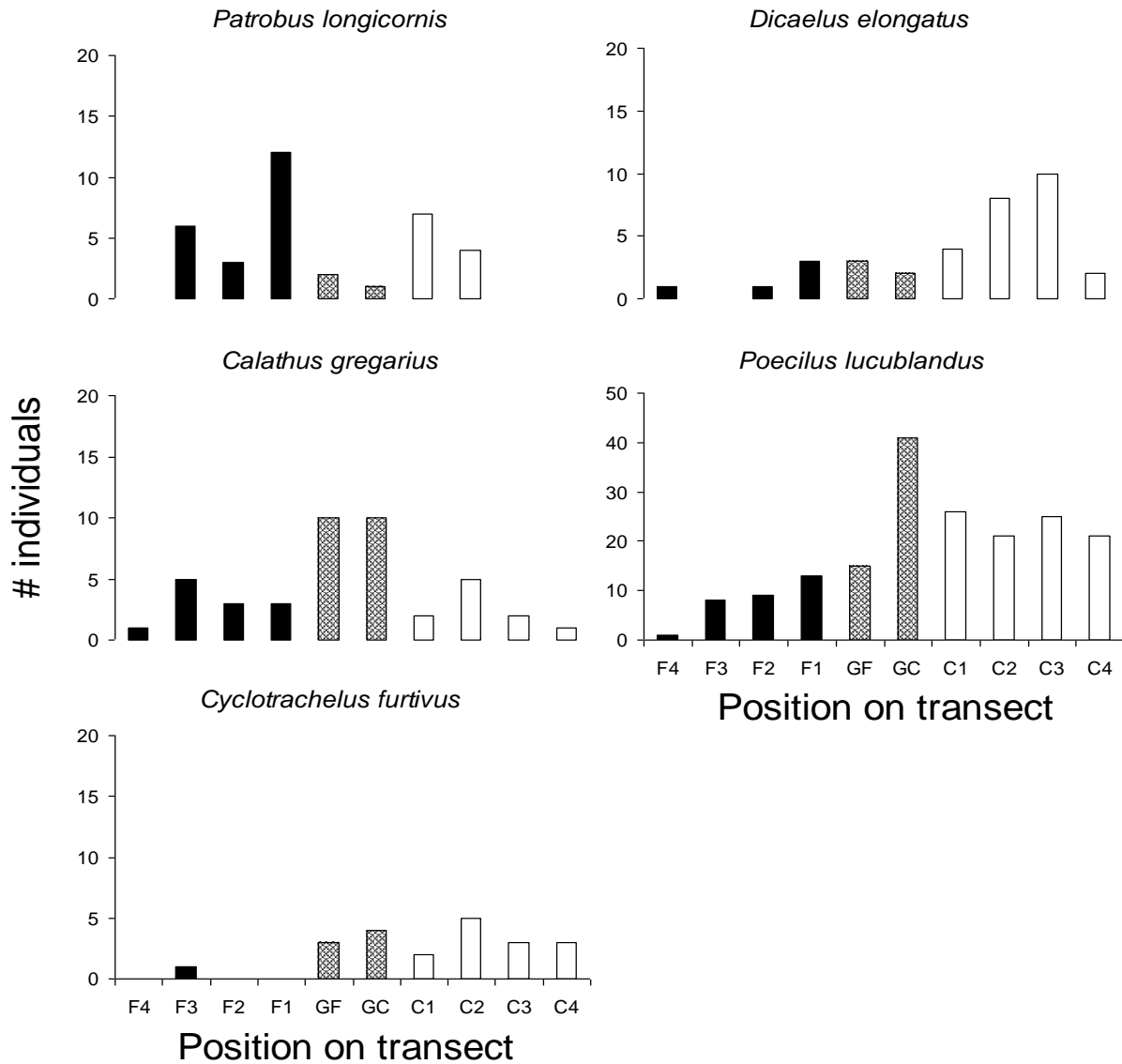


Figure 9. Distribution of four species of Carabidae along a transect extending from forest (F4-F1), through a mowed grassy margin (GF and GC), and into corn (C1-C4). Traps were 5m apart except for the distal traps (F4 and C4), which were situated 15m from the adjacent trap. Carabid species in this figure were found to be habitat generalists.

Chapter 6

Conclusions

Genetically-engineered (GE) crops have become a prominent fixture in U.S. agriculture, yet there is still not a consensus regarding the extent of their environmental impacts. However, within the realm of non-target effects, a clearer picture is developing for GE crops conferring insect resistance (i.e., *Bt* crops). Due to the insecticidal properties of *Bt* crops, there has been concern for possible direct and indirect effects to non-target organisms (Angle 1994, Groot and Dicke 2002). Overwhelmingly, evidence suggests that transgenes conferring the expression of *Bt* Cry toxins used in agriculture today are not directly toxic to most non-target organisms typically found within agricultural fields, although indirect effects have been noted in a multi-trophic context. Indirect effects to natural enemies may occur when fed prey that has been sublethally-compromised by *Bt* toxins (e.g., Hilbeck et al. 1998). However, this is due to the nutritional value of the prey and not direct toxicity (Dutton et al. 2002), and in a field setting where alternative prey is available this would be of less concern. Indirect effects have also been observed for specialist predators (Riddick et al. 1998) or parasitoids (Pilcher et al. 2005) due to lack of a particular prey species susceptible to *Bt* toxins. In these cases, the hosts were prominent agricultural pests targeted for control in most agricultural settings.

Our results suggest that dynamic shifts in insecticide use patterns and delivery systems due, in part, to the deployment of *Bt* crops may more likely influence non-target populations and communities through direct or indirect effects. This premise has been

supported by recent reviews (Romeis et al. 2006) and meta-analyses (Marvier et al. 2007). Our studies highlight two instances where such shifts in insect use and delivery patterns may affect non-target organisms: 1) a reduction in insecticide inputs due to the target specificity of *Bt* crops, which may sustain or promote insect diversity within crop fields, and 2) technologies that are inherently coupled with certain *Bt* crops (i.e., neonicotinoid seed treatments on *Bt* field corn) that may negatively affect non-target organisms.

The diversified vegetable study (Chapter 2) examined shifts in insecticide use stemming from the use of transgenic crops and effects to epigeal insect diversity. Transgenic and isoline diversified vegetable crops, consisting of sweet corn, potato and acorn squash, required different insecticide inputs based upon IPM pest thresholds and normal management procedures. Over 2 years, transgenic crops received fewer insecticide applications than the isoline crops. This difference was primarily due to the ability of the transgenic cultivars to effectively control the target pests.

Despite the differences in insecticide inputs, there were no detectable differences in insect species richness or dominance distribution between transgenic and isoline treatments in our study. In almost all cases, transgenic treatments had higher species richness estimates, however these differences were not significantly different. In regions of large crop acreages where pest pressure is greater, and insecticide inputs are used at much higher levels (e.g., Arizona cotton), the reduction of insecticide inputs due to the deployment of *Bt* crops, can result in noticeably higher levels of beneficial insect diversity (Cattaneo et al. 2006). Instead, our findings from smaller acreage farms in a heterogeneous landscape found that crop species, and crop rotations to an even larger

extent, had a stronger influence on epigeal insect communities. For example, carabids known to be weed seed predators were found in higher abundances in the the squash and sweet corn, where the open environment may have been more conducive to weed invasion and development. We concluded that, at least in the short term, the heterogeneity of crops coupled with crop rotation patterns common to diversified vegetable farms in northeastern U.S. agroecosystems seems to aid in preserving epigeal biodiversity, which may be supplemented by reductions in insecticide use associated with transgenic crops.

This general conclusion was supported throughout our other studies where transgenic maize was compared to conventionally-managed maize (Chapter 3-5). In all instances, no differences in species richness or dominance distribution were found between management regimes. When compared to a control treatment (no insecticide inputs), population- and community-level differences were observed, however even these effects were lost when crop rotations were employed. The idea of community legacy based on previous land use and the extent to which crop rotation acts as a species mixing tool warrant further investigation, especially in areas with diverse agricultural landscapes such as the northeastern U.S.

The field corn plots in the two-year maize mosaic study (Chapters 3 and 4) were not rotated and represented situations in which a community legacy could develop, making the results from the second year theoretically more reliable. Due to the large size of this experiment, the plots were initially situated over a previously diverse crop mosaic from the year preceding the experiment, which we hypothesized resulted in confounding community legacy effects influencing the first year of the experiment. Indeed, only in

second year field corn did we obtain results indicating that coleopteran populations and communities were affected by both transgenic and conventional management inputs, when compared to a control receiving no insecticides. It is important to note that the transgenic field corn contained a neonicotinoid seed treatment that likely had more of an influence on non-target coleopterans than the Cry toxins. In a laboratory study performed in conjunction with our field studies, Mullin et al. (2006) identified neonicotinoid seed treatments found on Cry3Bb transgenic field corn as a major direct mortality factor for carabids, and not the Cry3Bb toxins.

At the population level, one of the most abundant carabid species, *Harpalus pensylvanicus*, was found at significantly higher abundances in the control than in the treated fields. All insecticide management inputs (i.e., neonicotinoid seed treatment in the transgenic corn, or an at-planting pyrethroid soil application in the conventional corn) occurred early in the season when the less-mobile *H. pensylvanicus* larvae would be feeding and in close proximity to the corn seed in the soil. The possible insecticide-induced mortality factors seem to be evident in the trap captures of adults later in the season. When examining the entire carabid community, no difference in species richness was found between the treatments, however the activity-density (total # of carabids collected) was nearly double in the control plot compared to the treated plots in the second year.

Ordination analyses indicated the presence of distinct community gradients among the coleopteran community (represented by the most abundant Carabidae, Chrysomelidae, and Nitidulidae) in the second year field corn. Community differences were primarily characterized by the control community differentiating from the treatment

communities. Although longer-term studies are needed, early indications are that non-target herbivores may be affected by Cry proteins coupled with systemic neonicotinoids found on transgenic field corn seed, or by the soil insecticides applied in the conventional fields. Results for carabids and nitidulids varied between species, however the tendency was for higher activity-densities in the control treatment.

PRC analyses examined community shifts over time and suggested that transgenic and conventional management effects on non-target coleopterans were indistinguishable, yet both showed a transitory deviation from the control. This is most likely due to the presence of the neonicotinoid seed treatment associated with the transgenic seed, since there is no evidence that Cry proteins are directly affecting non-target organisms. By comparison, in the second year sweet corn, which was rotated, no differences among coleopteran communities could be discerned. Our work suggests coleopteran community-level effects are more apparent after multiple years of non-rotated management; thus these effects from systems using coupled transgenic-neonicotinoid technology warrant further study in longer-term experiments.

The results of the first two studies suggested that habitat modification (i.e., crop species and crop rotations) may exert forces equal to or greater than pest management inputs on non-target epigeal insects. In the final study (Chapter 5) we expanded our collection area and examined the influence of the surrounding habitat on Carabidae communities within crop fields, by deploying transects of traps extending from corn, through a grassy margin, and into a forest edge. At each trap location we also measured plant diversity and structure characteristics.

Carabidae diversity measurements were highest in the forest, and grass to a lesser extent, where plant diversity measurements were also higher. It is difficult to determine whether this bottom up influence is driven by the taxonomic diversity of plants or the increase in structural complexity (i.e., more microhabitats) that they represent, since both are highly correlated. There is increasing evidence that vegetation structural diversity plays a larger role in supporting carabid diversity, than does plant taxonomic diversity (Brose 2003). In our findings, plant species richness and diversity were highest in both the forest and the grass, however vegetation structure was different between the two habitats. Since carabid species richness estimates were highest in the forest, this suggests that vegetation structure or level of disturbance (i.e., grass was mowed) may play an important role in defining carabid diversity. In the corn, where carabid species richness was the lowest, the vegetation structure associated with the carabids ground-dwelling habitats (i.e., % litter and % ground cover) were also the lowest. However, activity-density of carabids was high, as the open environment was conducive to hunting and arguably the weed seed load would also be higher in corn.

Most of the abundant carabids displayed a strong affinity for particular habitats and community shifts along the transect were most rapid in the grassy margin between the forest and the corn. However, nearly all of the abundant carabids were found in more than one habitat, and most were found in all three. Some carabids utilize multiple habitats for reproductive, overwintering, or feeding purposes, and the sharing of species between habitats has been documented elsewhere (Bedford and Usher 1994, Dennis et al. 1994, French et al. 2001). From this study, we identified several species as habitat generalists that seemed to benefit from the forest edge. We also found several species that were

strongly associated with the forest, yet were still found in substantial numbers within the nearby cornfield.

The influence of the forest edge on the carabid communities in corn were investigated at the landscape level by comparing the carabid communities in corn near the forest edge (i.e., forest-near corn) with the communities collected in sweet corn and field corn from our other experiments (Chapter 2-4). The others studies were located nearby, but at a farther distance from the forest where the surrounding habitat was primarily agricultural fields (i.e., forest-far corn). Our findings supported the notion that landscape-scale factors are influential in defining local insect diversity. Based on shared-species similarity indices, carabid communities in forest-far corn locations were consistently more similar to each other than with the carabid community found in the forest-near corn. While the shape of the dominance distribution was similar in both areas, the dissimilarity between forest-near corn and forest-far corn was driven by shifts in the relative abundance of shared species and the acquisition of new species that favor the forest edge habitat.

Despite the apparent influence of the forest edge on carabid community composition in the corn, it is yet unclear whether this represents simply a community shift (in comparison to forest-far corn), or an actual increase in diversity within agroecosystems at the forest edge location. Future work in this area would be warranted, as there is increasing evidence of a positive relationship between biodiversity and ecosystem function (Cardinale et al. 2006), and when considering multiple ecosystems functions of species (as is often the case in agriculture), even higher levels of biodiversity may be needed (Hector and Bagchi 2007). These positive relationships are found to be

asymptotic due to functional redundancy, however this redundancy can act as a “buffer”, adding stability to an ecosystem (McCann 2000, Tilman et al. 2006).

The work represented in this thesis greatly benefited from our focus on species level identification. While species-level identification often requires large investments of time and money, which can be exacerbated by the bottleneck in taxonomic expertise (Kim and Byrne 2006), we have defined an approach that focuses on a functionally diverse subset of the non-target community in question, for which species-level taxonomic resolution can be achieved. This allows for the greatest level of biological and ecological inference. The benefits from such an approach would be lost in studies focusing on higher-level taxa, such as families. For example, throughout our studies we found that different carabid species responded differently to farm management practices. Likewise, in the ecotone study, several species were identified that benefited from the forest edge and may contribute to diversity within adjacent crop fields. With species level identification we were also able to investigate biodiversity measures such as species richness and evenness, and compare the dominant community members between treatments and habitats.

Species level data also allowed ecologically relevant analyses of the population dynamics of dominant species. In chapter 3, we found temporal partitioning of activity-densities of adults, which may help explain co-existence of multiple species in the same habitat. Insects, as a group, can be hyper-diverse, and temporal partitioning is one mechanism for securing resources while achieving species-packing. Where dominant species overlapped temporally, there was evidence, based on known feeding preferences, that they segregated functionally.

Our species lists and the preservation of voucher specimens also serve as valuable baseline data on epigeal insect communities in central PA agroecosystems. Over the course of the study we identified 108 carabid species (Appendix B) of which many were new county records. Within a crop in a single year, we often found over 40 carabid species, an amount higher than has generally been recorded (~ 30 species) in other temperate growing regions (Luff 2002). In addition to Carabidae, the numerous other species identified and curated throughout our work, represent many groups for which there is little information (e.g. Staphylinidae) and will undoubtedly be useful for future studies in the area.

Although agriculture represents managed lands which can adversely affect biodiversity and non-target organisms, our findings portray a quite diverse community of insects found in northeastern U.S. agroecosystems, that in some instances was greater than documented in other temperate growing regions. The adoption of transgenic crops within these agroecosystems may promote or sustain this diversity if they substantially reduce insecticide use compared to conventional practices. On the other hand, transgenic crops that are accompanied with other pest management technologies, such as seed treatments, may have effects on non-target organisms that are indistinguishable from conventional management practices. Regardless of these impacts, our results suggest that the diversity of northeastern U.S. agroecosystems at multiple spatial (diversified cropping systems, field margins, landscape heterogeneity) and temporal (crop rotation practices) scales seems to promote on-farm insect diversity. Ultimately, an increased understanding of these patterns will better inform farm and landscape management decisions that can

support insect diversity and ecosystem function, as part of more sustainable pest management programs.

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APPENDIX A

Coleopteran families (other than the Carabidae, Chrysomelidae, and Nitidulidae shown in Table 4.2) and species collected in maize at Penn State research Farms in Rock Springs, PA during 2003 and 2004.

ANTHICIDAE	CLAMBIDAE	LANGURIIDAE
<i>Acanthinus myrmecops</i>	COCCINELLIDAE	<i>Toramus</i> sp.
<i>Anthicus cervinus</i>	<i>Coccinella septempunctata</i>	LEIODIDAE
<i>Anthicus virginiae</i>	<i>Coleomegilla maculata</i>	<i>Anistoma blanchardi</i>
<i>Malporus formicarius</i>	<i>Harmonia axyridis</i>	MELYRIDAE
<i>Notoxus anchora</i>	<i>Hippodamia convergens</i>	<i>Collops quadrimaculatus</i>
<i>Notoxus desertus</i>	<i>Hippodamia paranthesis</i>	<i>Collops vitatus</i>
<i>Tomoderus constrictus</i>	CORYLOPHIDAE	MORDELLIDAE
BYRRHIDAE	<i>Sericoderus</i> sp.	PHALACRIDIDAE
<i>Byrrhus americanus</i>	CUCUJIDAE	<i>Acylopus ergoti</i>
<i>Curimopsis</i> sp.	<i>Telephanus velox</i>	<i>Stilbus apicalis</i>
<i>Cytilus alternatus</i>	CURCULIONIDAE	<i>Stilbus nitidus</i>
<i>Simplocaria semistriata</i>	DERMESTIDAE	SCAPHIIDAE
CANTHARIDAE	ELATERIDAE	<i>Baeocera</i> sp.
<i>Chauliognathus marginatus</i>	<i>Aeolus dorsalis</i>	SCARABAEIDAE
<i>Podabrus modestus</i>	ENDOMYCIDAE	<i>Popilla japonica</i>
<i>Podabrus rugosulus</i>	HISTERIDAE	<i>Serica sericea</i>
CEPHALOIDAE	HYDROPHILIDAE	STAPHYLINIDAE
CERAMBYCIDAE	<i>Helophorus</i> sp.	
<i>Tetraopes tetrophthalmus</i>	LAMPYRIDAE	

APPENDIX B

Table 1. Carabidae collected in Rock Springs, PA over five years of field collection.

Asterisks (*) indicate new county records for Centre County, PA

<i>Agonum cupripenne</i>	<i>Bradycellus rupestris</i>	<i>Notiophilus aeneus</i>
<i>Agonum ferreum</i>	<i>Bradycellus tantillus</i>	<i>Notiophilus ceneus</i>
<i>Agonum melanarium</i>	<i>Calathus gregarius</i>	<i>Olisthopus parmatus</i>
<i>Agonum muelleri</i>	<i>Chlaenius emarginatus</i>	<i>Oodes amaroides</i>
<i>Agonum palustre</i>	<i>Chlaenius tricolor tricolor</i>	<i>Patrobus longicornis</i>
<i>Agonum placidum</i>	<i>Cicindela punctulata punctulata</i>	<i>Platynus decentis</i>
<i>Agonum punctiforme</i>	<i>Cicindela sexguttata</i>	<i>Poecilus chalcites</i>
<i>Agonum retractum</i>	<i>Cicindela tranquebarica</i>	<i>Poecilus lucublandus</i>
<i>Amara aenea</i>	<i>Clivina bipustulata</i>	<i>Pseudamara arenaria</i>
<i>Amara apicaria</i>	<i>Clivina impressifrons</i>	<i>Pterostichus adoxus</i>
<i>Amara cupreolata</i>	<i>Colliuris pensylvanica</i>	<i>Pterostichus caudicalis</i>
<i>Amara exrata</i>	<i>Cyclotrachelus furtivus</i>	<i>Pterostichus commutabilis</i>
<i>Amara familiaris</i>	<i>Cymindis limbata</i>	<i>Pterostichus coracinus</i>
<i>Amara impuncticollis</i>	<i>Cymindis platicollis</i>	<i>Pterostichus luctuosus</i>
<i>Amara littoralis</i>	<i>Cymindis cribricollis</i>	<i>Pterostichus melanarius</i>
<i>Amara musculus</i>	<i>Dicaelus elongatus</i>	<i>Pterostichus mutus</i>
<i>Amara ovata</i>	<i>Dicaelus politus</i>	<i>Pterostichus pensylvanicus</i>
<i>Amara rubrica</i>	<i>Diplocheila obtusa</i>	<i>Pterostichus rostratus</i>
<i>Amphasia interstitialis</i>	<i>Dyschirius globulosus</i>	<i>Pterostichus stygicus</i>
<i>Anisodactylus harrisii</i>	<i>Elaphropus anceps</i>	<i>Pterostichus tristis</i>
<i>Anisodactylus melanopus</i>	<i>Harpalus affinis</i>	<i>Scarites quadriceps</i>
<i>Anisodactylus nigerrimus</i>	<i>Harpalus caliginosus</i>	<i>Scarites subterraneus</i>
<i>Anisodactylus nigrita</i>	<i>Harpalus compar</i>	<i>Sphaeroderus stenostomus</i>
<i>Anisodactylus rusticus</i>	<i>Harpalus erythropus</i>	<i>Stenolophus comma</i>
<i>Anisodactylus sanctaerucis</i>	<i>Harpalus faunus</i>	<i>Stenolophus conjunctus</i>
<i>Apenes lucidulus</i>	<i>Harpalus herbivagus</i>	<i>Stenolophus ochropezus</i>
<i>Badister notatus</i>	<i>Harpalus longicollis</i>	<i>Stenolophus rotundatus</i>
<i>Bembidion affine</i>	<i>Harpalus pensylvanicus</i>	<i>Stenolophus rotundicollis</i>
<i>Bembidion impotens</i>	<i>Harpalus protractus</i>	<i>Syntomus americanus</i>
<i>Bembidion mimus</i>	<i>Harpalus rubripes</i>	<i>Synuchus impunctatus</i>
<i>Bembidion obtusum</i>	<i>Harpalus somnulentus</i>	<i>Trechus quadrisignatus</i>
<i>Bembidion patruelle</i>	<i>Lebia viridis</i>	<i>Trichotichnus fulgens</i>
<i>Bembidion quad. opp.</i>	<i>Microlestes linearis</i>	<i>Trichotichnus autumnalis</i>
<i>Bembidion rapidum</i>	<i>Microlestes pusio</i>	<i>Trichotichnus dichrous</i>
<i>Bembidion versicolor</i>	<i>Notiobia nitidipennis</i>	<i>Trichotichnus vulpeculus</i>
<i>Bradycellus lugubris</i>	<i>Notiobia terminata</i>	<i>Xestonotus lugubris</i>

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PEER-REVIEWED PUBLICATIONS

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