ABSTRACT

Title of Dissertation: GROUND-DWELLING BEETLES AS BIOINDICATORS IN TRANSGENIC CORN

Miles David Lepping, Doctor of Philosophy, 2009

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Ecological risk assessment for transgenic crops requires identification of appropriate biological indicator organisms for use in laboratory and field biomonitoring studies. Ground-dwelling predatory beetles in the families Carabidae and Staphylinidae comprise a diverse and abundant group of nontarget organisms in field corn systems where rootworm-resistant transgenic varieties are deployed. First, the utility of two sampling methods (pitfall trapping and suction-based litter extraction) was assessed for estimating ground beetle (Coleoptera: Carabidae) population parameters in Maryland cornfields. Sampling bias was established for pitfall trapping, confirming the limitations of this semi-quantitative method for capturing a representative portion of the epigeal community. Litter extraction data conformed to predictions for abundance in relation to trophic identity, body size and biomass. Litter extraction identified smaller bodied carabid omnivores and carnivores as numerically dominant over larger bodied species

that have received focus in risk assessment studies. A small-bodied carabid, *Elaphropus xanthopus* (Dejean), was identified as the dominant carnivore, and therefore selected for nontarget exposure and toxicity studies. Second, in choice and no-choice experiments, corn pollen was identified as a realistic, direct exposure pathway to transgenic proteins for *E. xanthopus*. Third, organism-level exposure to Cry34Ab1 rootworm-resistant protein was demonstrated for *E. xanthopus* in the laboratory and field during corn pollen shed. Field studies also revealed contamination across transgenic and non-transgenic test plots, indicating experimental design must account for the movement of study organisms and/or transgenic plant tissues. Finally, a toxicity study examined the effects of dietary exposure to rootworm-resistant Cry34/35Ab1 corn pollen for two beetle species, a carabid, *E. xanthopus*, and a staphylinid, *Strigota ambigua* (Erichson). Transgenic pollen exposure did not affect longevity or sub-lethal behaviors for either species. Small-bodied, predatory ground beetles are recommended as candidate bioindicator organisms in risk assessment studies designed to optimize field monitoring, exposure detection, and bioassay for transgenic pesticides.

GROUND-DWELLING BEETLES AS BIOINDICATORS IN TRANSGENIC CORN

By

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Dissertation submitted to the Faculty of the Graduate School of the
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Shortly thereafter, Paula joined the faculty at Maryland and has since been my primary advisor and champion throughout my academic career. Her outstanding abilities as a mentor are evidenced by her students' successes, and her dedication to my pursuits has been particularly selfless as my research broadened from the green industries into agriculture. Paula provided me the opportunity and autonomy to build a research program that merged ecology and microbiology, thus increasing my exposure to multiple disciplines and creating additional opportunities to train under an umbrella of expertise. At every turn, Paula has straightened the path. Her grace and good humor have accented her phenomenal mentoring and ameliorated the more challenging aspects of research. I have been one of the fortunate few to witness the establishment and growth of Shrewsbury Lab - a family whose bonds are as strong as our laughs are loud and long. Paula's lab is a welcoming home and proving ground for our ideas, and will always be an environment I aim to recreate.

An interest in nontarget organisms naturally led me to Galen Dively, who kindly accepted an invitation to join my committee and in short order generously offered me grant support and flexibility in selection of research trajectory. The community spirit in our department has been exemplified by the open door and busy hive of Dively Lab – the beacon of answers. Perhaps the only professor I've ever met whose desk is in the lab, Galen has made himself available to everyone, and provided or facilitated answers to just about every question. Galen's ingenuity has reinvented the field of nontarget risk assessment. In this process he has shared his strategies and tools in a way that benefits everyone across the science and launches each of us closer to our goals. Galen has guided and inspired me to apply his methods to every facet of research. His affable and approachable personality has brought us all closer to a place where we share in his love of adventure.

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General Introduction

Legal marketing of a pesticide in the United States requires registration with the U.S. Environmental Protection Agency (EPA), which serves as the regulatory and enforcement body for any chemical intended to "prevent, destroy, repel or mitigate any pest" (target organism) (40 C.F.R. § 152.3). Requirements for EPA registration often include nontarget organism (NTO) testing to assess the risk to non-pest organisms and the potential for disruption of ecological function if a novel pesticide is deployed into the environment. The term *nontarget organism* broadly refers to any organism hypothetically exposed to a pesticide that is not listed for control on the EPA label.

Nontarget studies examining the potential for and effects of pesticide exposure are conducted within the framework of ecological risk assessment (ERA). In ERA, risk is characterized through hazard identification, exposure assessment, and dose-response relationships. Nontarget effects are defined as negative consequences due to pesticide exposure. Negative consequences due to intoxication may include weakened performance (e.g. lower survival) and reduced fitness (e.g. lower fecundity). Nontarget organisms of interest are historically those which are considered beneficial, in that they perform ecosystem services such as pest population regulation, nutrient cycling, pollination, or are representative of key groups targeted for conservation of biodiversity (e.g. endangered species).

Nontarget testing is guided by a tiered (hierarchical) system which ranges from short-term laboratory studies (Tier I) to field studies (Tier IV). If adverse effects are detected following lower tier studies, or there are reasonable concerns, government regulators may warrant higher tier studies in order to adequately characterize the risk. In Tier I tests, pre-selected "benchmark" biological indicator organisms (bioindicators) are exposed to high doses of purified toxins in order to screen for overt negative effects (e.g. worst-case scenarios). Tier I bioindicators are intended to represent the taxonomic groups to which they belong, and have often been found sensitive to characterized toxins. The EPA has defined preferred bioindicators for Tier I studies at the species-level for several taxonomic groups (e.g. mammals, birds, freshwater fish, estuarine and marine invertebrates) (US EPA 1996a-d). However, with the exception of honeybees (*Apis mellifera* L.) (US EPA 1996f), insect bioindicators are not defined, allowing scientists to choose taxa on a case-by-case basis. Currently, EPA suggests, but does not require, the use of the following insect groups in Tier I tests: predaceous coleopterans (beetles), parasitic dipterans (true flies), predaceous hemipterans (true bugs), parasitic hymenopterans (wasps), and predaceous neuropterans (net-winged insects) (US EPA 1996e).

With the advent of genetic transformation technology, genes coding for pesticidal substances originating from a donor organism may be incorporated into the unrelated genome of a recipient organism (e.g. agricultural plant), producing a genetically modified organism (GMO). Following transformation, the resulting transgenic plant (genetically modified [GM] / genetically engineered [GE] plant) may then express the novel trait(s)

coded for by the donor organism's genes. The present research examines transgenic plant traits that confer resistance to insect pests. Genetically integrated toxins are commonly termed *plant-incorporated protectants* (PIPs), transgenic *plant protection products*, and often truncated to *transgenic toxins*. The transgenic crop plants of interest here are those which express proteins derived from a ubiquitous bacterium, *Bacillus thuringiensis* (Bt) (Berliner) (Bacillales: Bacillaceae). As a group, Bt strains are toxic to a wide range of insect taxa (Bravo 1997), although individual Bt crystalline (Cry) proteins exhibit more narrow activity. First generation Bt plants targeted caterpillar pests in the order Lepidoptera (Vaeck et al. 1987). Shortly thereafter, varieties designed to target beetle pests in the order Coleoptera were developed. Since transgenic pesticide production is based on the insertion of foreign genes into recipient organisms, the potential for unintended effects (e.g. nontarget effects, gene out-crossing with wild relatives, pest resistance) are investigated. Public and private sector concern over issues regarding food safety of GMOs has prompted higher tiered studies despite the absence of findings in lower tiers. For Bt plants, no direct nontarget effects have been revealed to date, although indirect effects have been observed. For example, reductions in predator and parasitoid populations may be due to the loss of primary herbivore pests (prey/hosts) in Bt cropping systems (Dively 2005, Naranjo 2005). Nonetheless, transgenic plant scrutiny has increased due to high profile food contamination issues (Bucchini and Goldman 2002), out-crossing (Mallory-Smith and Zapiola 2008), and studies concluding nontarget effects (Hilbeck et al. 1998a/b, Losey 1999, Lövei et al. 2009, Schmidt et al. 2009, see also Gatehouse et al. 2002, Dively et al. 2004, Rauschen 2009, Shelton et al. 2009). In the

nontarget arena, regulatory science and policy is currently undergoing substantial reform (Rose 2007, Romeis et al. 2008, Todd et al. 2008).

Nontarget studies are shifting from testing transgenic toxins against organisms outside of the target order to species more closely related to the target taxa. Two of the most recent Bt proteins (Cry34Ab1 and Cry35Ab1) are expressed together in field corn as a binary toxin (Cry34/35Ab1) (US EPA 2005); designed to target root-feeding beetle larvae in the genus, *Diabrotica* (Coleoptera: Chrysomelidae). Expression of Bt toxins in the cropping environment may result in exposure for nontarget predaceous coleopterans. One of the most diverse and numerically dominant groups in the epigeal predator guild includes ground-dwelling beetles in the family Carabidae. Of the few nontarget studies examining carabids at the species-level, most have focused on large-bodied species purported to be dominant predators in agroecosystems. To complement the existing literature, GMO nontarget studies are needed to assess small-bodied predaceous carabids, which may be more appropriate biological indicator organisms based on abundance and likelihood of pesticide exposure. Simultaneously, few studies have quantified ground-beetle exposure to transgenic toxins (Peterson et al. 2009), or incorporated realistic exposure pathways in toxicity studies. Finally, building on the wealth of information provided by carabidologists, species-level ecology is needed to develop profiles that may assist interpretation of population changes should they occur following exposure to future transgenic plant toxins.

In summary, current data gaps in nontarget testing of ground-dwelling beetles include: (1) the selection of appropriate nontarget species for tiered testing; (2) examination of small-bodied, potentially more abundant ground-dwelling beetle species; (3) quantification of Bt protein exposure in the laboratory and field; (4) toxicology studies incorporating realistic exposure pathways to transgenic toxins; and (5) lack of basic description of ecosystem function. The following research addresses these issues.

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CHAPTER 1: Bioindicator selection for Carabidae in agroecosystems: estimating population density and structure

Abstract

Within ecological risk assessment programs examining conventional and transgenic pesticides, there is need for appropriate biological indicators from the ground beetle family, Carabidae. Concurrently, identification of candidate bioindicators requires a sampling tool that provides an accurate representation of carabid diversity and abundance. Field studies monitoring carabid beetle populations often utilize semi-quantitative methods (e.g. pitfall trapping) to estimate diversity and absolute densities. While pitfall trapping and related techniques are convenient, they do not produce standardized data (number of individuals per unit area) for comparison to other studies and are susceptible to intrinsic sampling biases that may underestimate common arthropod groups. In combination with a G-vac (leaf-blower/vacuum), a novel suction sampler collection unit is described that enabled high-throughput litter extraction of carabid adults and other hard-bodied taxa. Litter extraction using the modified G-vac was compared to pitfall trapping to examine tradeoffs and utility as complementary methods. Ground beetle populations were monitored in Maryland field corn during three cropping events (late whorl/canopy close, anthesis, post-harvest) to screen taxa for use as bioindicators. Carabid beetles were point sampled during day and night periods using litter extraction and continuously sampled using pitfall traps for two week periods surrounding each cropping event. Sampling time-of-day did not influence litter extraction estimates of species composition, suggesting differences in species activity may not

affect species detection. Species capture efficiency differed significantly between litter extraction and pitfall trapping, and consequently produced conflicting results between sampling methods for: (1) species dominance as a function of abundance, biomass, and trophic identity; (2) relationships of abundance versus body length or mean individual biomass (MIB); and (3) study site selection. For bioindicator candidacy, pitfall trapping data confirmed the selections by previous studies for common, large-bodied omnivores (e.g. *Harpalus pensylvanicus*). In contrast, litter extraction identified small-bodied omnivores in the genera *Stenolophus* and *Amara* as dominant species in terms of abundance and biomass, respectively. Litter extraction also identified small-bodied carnivores (e.g. *Elaphropus* spp.) as numerically dominant over large-bodied carnivorous species that have been selected for nontarget testing in previous studies. Small-bodied carabids may be more appropriate candidates for biomonitoring studies based on abundance and the concomitant potential for exposure to pesticides. Suction sampler-based litter extraction is suggested as a primary sampling method for use in biomonitoring studies, as: (1) carabid species richness and density (number of individuals per unit area) are rapidly estimated; (2) resulting data conform to normative expectations for density versus body size, biomass, and trophic identity; and (3) density estimates allow direct comparisons within and between studies.

Key Words: body size, dominance structure, ecological risk assessment, pitfall trapping, suction sampling

Introduction

Biological indicator species (bioindicators) are identified based on many definitions of their utility for documenting ecological and environmental change (McGeoch 1998). At the scientific level, comprehensive bioindicator selection includes satisfaction of criteria specific to laboratory and field experimentation. In addition to being hypothetically exposed (e.g. nontarget organism), bioindicator selection criteria may include:

1) sensitivity to environmental change or stressor of interest (bioindication);
2) exposure to environmental stressor of interest;
3) accessibility for monitoring (readily sampled, sorted and identified);
4) representative of focal community, taxonomic or functional group;
5) habitat- or site-specificity;
6) high abundance and even distribution within site (fidelity);
7) defined ecological function;
8) human valuation (e.g. biodiversity, ecosystem service provider);
9) economy of monitoring; and
10) feasibility of captive rearing (Tier I testing);

(Pearson 1994, Dufrêne and Legendre 1997, McGeoch 1998, McGeoch and Chown 1998, Andersen 1999, Kerr et al. 2000, Döring et al. 2003, Duelli and Obrist 2003, Niemi and McDonald 2004, Romeis et al. 2006). The present chapter addresses criteria 3 through 6. Subsequent chapters address criteria 1 (Chapter 4), 2 (Chapter 3), 7 and 8 (Chapter 2).

The goal of biological monitoring (biomonitoring) is to detect and quantify population change in relation to environmental gradients. Selection of bioindicator species and biomonitoring depends on the reliability of sampling methods and validation of results given the absence of novel environmental stressors. If sampling methods introduce bias that is not uniform and directional then it may not be fully corrected in analysis. Additionally, failure to accurately distinguish a population metric estimate (signal) from natural variability (noise) may reduce study sensitivity and description of the focal community (Carignan and Villard 2002). To facilitate the examination of sampling method bias, normative expectations are defined as patterns of population structure (e.g. numerical dominance) that are constructed from observations recorded across multiple study systems and modes of sampling. Significant deviations from normative expectations suggest variation has been introduced into a study and must be accounted for in order to present unbiased results. In the present study, the influence of sampling method on bioindicator identification, as it relates to elucidating population density and structure for nontarget organisms, is examined in Maryland field corn systems.

In a variety of natural and managed systems, ground beetles (Coleoptera: Carabidae) have been utilized as bioindicators of habitat quality and environmental disturbance (Maelfait et al. 1990, Luff 1996, McGeoch 1998, Kromp 1999, Rainio and Niemelä 2003). Recently, carabid beetles have received focus in risk assessment programs examining the effects of transgenic toxins in agricultural systems (French et al. 2004, Ahmad et al. 2005/2006, Dively 2005, Zwahlen and Andow 2005, Harwood et al. 2006, Toschki et al. 2007, Floate et al. 2007, Leslie et al. 2009). Virtually all studies examining

ground-dwelling beetles as nontarget organisms or biological indicators have relied on semi-quantitative sampling methods, specifically use of the pitfall trap (Marvier et al. 2007). Limitations and biases of semi-quantitative and quantitative methods for estimating carabid population measures are briefly discussed. A formal review of these methods is presented by Sunderland et al. (1995).

Sampling methods

Among sampling methods, pitfall trapping has been the most commonly employed technique (Holland 2002), due to its ease of deployment, continuous trapping, and low cost. The basic pitfall trap is a plastic cup (or other vessel) sunk into a pit in the ground, with the rim level to the soil surface. Organisms unable to detect the presence of the trap fall in and are captured alive or die in a preservative liquid. Pitfall traps estimate *activity density* (formerly activity-abundance; see Woodcock 2005) – the interaction of absolute density and species movement. True densities are not estimated as the open (i.e. unfenced) pitfall trap does not trap organisms for any defined area (i.e. semi-quantitative). Therefore, "active species" (e.g. mobile but low agility) are likely caught in disproportionately greater numbers than relatively inactive or low density species (Mitchell 1963, Greenslade 1964, Luff 1975, Adis 1979, Sunderland 1988, Tonhasca 1993, Mommertz et al. 1996).

While activity density is partly dependent upon population density, pitfall trap catch may not reflect the absolute density of organisms (Briggs 1961, Adis 1979, Topping and Sunderland 1992, Spence and Niemelä 1994). In some cases, correlation between pitfall

trap catches and actual population densities may be weak or totally absent (Lang 2000). Moreover, activity density is often negligently applied as a surrogate for absolute density, which is more appropriately estimated using true quantitative methods. Pitfall trapping is also assumed to efficiently estimate species richness for primarily ground-dwelling species, a fallacy partly addressed by Luff (1996). Pitfall trapping results are influenced by direct effects of environment on carabid activity as well as species-by-trap interactions that are dictated by species characteristics and trap design (Appendices A and B). While environmental factors may be of interest, species-by-trap interactions are generally undesirable as they may skew the resulting description of the community under study.

For identifying appropriate bioindicators of toxin exposure, body size related factors are of particular interest. Body size is considered an important factor in determination of pitfall catch (Mommertz et al. 1996, Lang 2000). Studies have concluded that larger species are over-represented in pitfall catches (Spence and Niemelä 1994, Ulber and Wolf-Schwerin 1995, Lang 2000, Lin et al. 2005). Luff (1975) noted that smaller taxa may elude pitfall traps by way of differences in species ability to perceive the trap edge (Halsall and Wratten 1988), which suggests species richness and abundance have been underestimated for smaller taxa. Independent evidence supporting this hypothesis is found in over-wintering populations of adult carabids, where quantitative estimates (area-based unit of measure) revealed densities an order of magnitude higher for small-bodied groups (e.g. *Bembidion*) over larger bodied taxa (Geiger et al. 2009). While smaller diameter pitfall traps (Luff 1975, Work et al. 2002) and better designs (Luff 1996, Woodcock 2005) can catch small taxa, use of multiple traps sizes in biomonitoring

studies may increase study complexity without the benefits of an additional mode of capture. Finally, pitfall trapping may result in depletion effects (trapping out) of highly active carabids (Digweed et al. 1995), potentially resulting in local extinction and exclusion of focal populations for study. Poor correlation between pitfall trap capture and actual densities (Lang 2000), combined with species specific interpretations for many taxa (Spence and Niemelä 1994), suggests pitfall trapping is not the optimal tool for approximating population parameters for several taxa simultaneously.

Alternatively, determining the absolute density for carabids based on a defined soil surface area, although preferred (Morris 1960), is heavily dependent upon the efficiency tradeoff between sampling effort and efficacy, as low carabid numbers within small sampling areas or heterogeneous distributions (Greenslade 1964) may result in too little data to analyze. Absolute sampling for carabid beetles is most closely approximated with the quadrat method, where a defined area or volume (e.g. soil core; Edwards 1991) is thoroughly searched and debris is sorted in order to extract the desired life-stages. However, thorough quadrat sampling may be the most laborious of all methods (Dinter 1995), and is still subject to error and bias associated with the method used to extract organisms from litter and soil (e.g. flotation, funnel based methods, litter washing, manual, sieving, soil drench). Extraction method efficacy must be considered to ensure all organisms of interest are included in the density measurement.

Among near-absolute methods, suction-based sampling devices have also been used to estimate carabid densities. To date, the most common suction sampling device for

terrestrial arthropods is the Dietrick vacuum (D-vac) (Dietrick 1961). More contemporary suction sampling devices typically incorporate modified leaf-blower/vacuums (Garden vacuum or G-vac) (Bell at al. 2002, Bergthaler and Rlėys 2002). For epigeal arthropods (e.g. spiders and ground-dwelling beetles), modified G-vacs are superior to the D-vac (Stewart and Wright 1995). However, previous G-vac devices have included sample collection units typically consisting of a mesh bag fitted within the collection tube to accumulate samples. Sampling at or near ground level using a mesh-bag-in-tube configuration may result in soil and detritus accumulation within the collection tube to the point where suction and samples are lost. Toward a solution, Dively et al. (in prep.) conducted litter extraction sampling using a wet/dry canister vacuum – a device which couples strong suction and large sample capacity, but lacks high portability and design features that buffer organisms against the vacuum process. To address these issues, litter extraction in the present study includes a novel collection unit (see Materials and Methods).

Method comparison

Several method comparison studies have been conducted for pitfall trapping versus common alternatives (Appendices C and D), most of which suffer from either excessive labor requirements or marginally effective capture of carabid adults. These studies generally find that no one method is efficient for collection across the spectrum of ground-dwelling fauna (Sunderland et al. 1995). Specifically within Carabidae, there is little agreement between method pairings, suggesting coupling of quantitative and semi-quantitative techniques may always be necessary to fully parameterize carabid

populations. Nonetheless, the conclusion of several researchers (Morris 1960, Sunderland et al. 1995, Lang 2000) is that area-standardized estimates must be used to make any defensible statements about population "size", "abundance", or "density".

In an arthropod community study examining nontarget effects of conventional and transgenic pesticides, Dively et al. (in prep.) coupled pitfall trapping with litter sampling (both manual and canister vacuum). In terms of species composition, pitfall and vacuum captures in Dively et al. (in prep.) were different and likely influenced by beetle density and body size-by-sampling method factors. Comparison of manual- and vacuum-based litter extractions revealed manual extractions were more variable than canister vacuum extractions. The reduction in variance gained by the canister vacuum method suggests that this method may produce an adequate estimate of carabid population density across a large study site (e.g. high signal to noise ratio).

Capture efficiency

Adequate description of a community is directly influenced by the efficiency with which a sampling method captures a representative portion of the focal group in each sample. A fundamental measure of sampling method utility is *sampling method capture efficiency* (capture efficiency); broadly defined as the number of individuals captured divided by the total number of individuals present (Brook et al. 2008). The term, "capture efficiency" may also be applied to the accuracy of species detection relative to the total species richness of the focal group (species detected divided by total species richness). Accurate calculation of capture efficiency requires knowledge of the absolute density of target taxa

prior to sampling. In the absence of absolute density information, multiple methods may be compared to determine which methods capture a relative majority of the community, population, or species detected. Sampling methods that capture the most individuals and/or species may best estimate the group under study. In the present study, pitfall trapping would be expected to possess an overwhelming advantage in garnering more individuals and species as continuous trap deployment allows collection over multiple time points rather than the brief moment captured by a vacuum-sampled litter extraction.

Adult carabid size classes and biomass

In addition to traditional measures of community response for carabids (e.g. abundance, species richness and composition), body size and biomass have been examined to assess recovery following environmental pollution (Braun et al. 2004, Lövei and Magura 2006, Cárdenas and Hidalgo 2007), gradual changes due to management practices (Blake et al. 1994, Büchs et al. 2003), and urbanization (Niemelä et al. 2002, Weller and Ganzhorn 2004, Sadler et al. 2006). For example, Cárdenas and Hidalgo (2007) found that mean individual biomass (MIB) increased in a post-pollution recovery location for carnivorous but not for omnivorous carabids, suggesting differences in sensitivity to environmental stress and recovery of potential ecosystem services (e.g. weed seed or arthropod predation). Biomass has also been suggested for use in calculations of potential ecosystem impact by genetically modified organisms (Todd et al. 2008).

Previous biomonitoring studies examining body size or biomass have primarily relied on pitfall trapping to assess entire communities. Summarized from multiple studies and

sampling methods (e.g. insecticidal fogging, pitfall trapping), carabid body length ranges along a continuum from ≈0.7 mm (Erwin 1974) to ≈66 mm (Ball and Bousquet 2001) and carabid dry weight (xeromass) ranges from ≈0.3 to ≈440 mg (pitfall trapping; Jarošík 1989). In arable systems, carabid body length is typically between 3 and 24 mm, and dry weight may range from ≈0.16 to ≈160 mg (pitfall trapping, D-vac sampling, manual collection; Lang et al. 1997). Specifically for body size, the inherent biases of pitfall trapping reflected in previous studies indicate numerical dominance of larger-bodied species over smaller-bodied species. For example, reported body lengths for common carabids species were 9.9 mm for eastern Europe, and 7.8 mm for western Europe (weighted by species richness) (Lövei and Sárospataki 1990). Examination of carabids inhabiting hardwood forests in North America, Work et al. (2002) found that species with the highest activity densities included the body length range of ≈7 to ≈15 mm. Estimation of body size and biomass distributions using quadrat-based sampling methods is currently lacking in the literature for North American fauna, and may enable population pattern recognition with lower bias, and identification of more appropriate bioindicators.

Carabid species dominance

Species dominance is defined as the hierarchical order of species from the most abundant to the least abundant (e.g. species rank abundance). Accurate estimates of species dominance are necessary to establish the fundamental structure of a focal community. Summarizing conclusions based on pitfall trapping results, review articles describing carabid dominance patterns neither reach consensus nor fit expectations for body size or trophic identity (Lövei and Sárospataki 1990, Luff 2002).

A global observation is that as body size (and individual biomass) increases, abundance decreases (Currie 1993). This macro-ecological observation (normative expectation) serves as a baseline against which novel results may be compared. Comparing the dominance rankings for carabid taxa reported by Lövei and Sárospataki (1990) with the associated body sizes of those taxa, body size generally increases with increasing dominance rank. This trend runs opposite to the normative expectation cited above, where plots of abundance against body size reveal negative or arching relationships (e.g. triangular or polygonal) (birds: Nee et al. 1991, Greenwood et al. 1996; fish: Cohen et al. 2003; insects: Gaston and Lawton 1988, Stork and Blackburn 1993; mammals: Blackburn and Gaston 1997). Within Insecta, plots of abundance against body size often reveal an arching response where intermediate body sizes are most abundant, perhaps revealing optimal body size ranges (Siemann et al. 1999). While the most dominant carabid species reported by Lövei and Sárospataki (1990) are intermediate in size with respect to the spectrum of carabid body sizes in arable systems (3 to 24 mm) (Lang et al. 1997), these findings are still based on a semi-quantitative method (pitfall trapping), and therefore may provide more insight into the body sizes of speciose genera (e.g. optimal body sizes) rather than an accurate representation of species abundance and body size patterns. In an insect community study examining relationships between abundance, diversity and body size, Siemann et al. (1999) examined a broad range of insect orders using the semi-quantitative techniques of sweep netting and pitfall trapping. While they did not show detail for Carabidae, abundance versus body size plots for ground-dwelling fauna revealed the expected arched response, where intermediate sizes were most abundant, and

the smallest and largest body sizes were not (Siemann et al. 1999). A similar arched response is expected for body size in Carabidae, although the question of body size distribution (skew) should be addressed using quantitative methods. Significant deviations from the normative expectations for abundance and body size may suggest that the sampling method in question does not provide an accurate representation of the community under study.

Abundance versus body length and biomass

The following example summarizes the underlying conflict between prediction and observation for pitfall trapping when examining body size-abundance relationships. The energetic equivalence rule (EER) (Damuth 1981), predicts that the amount of energy used by species should be independent of body size. This rule is derived from empirical data which describes the relationship between population density (D) and body size (x) as $D \propto x^{-0.75}$ and from the allometric law relating metabolism (B) to body size (x) through the relationship, $B \propto x^{0.75}$. Calculating energy use (E) as a product of population density and metabolism ($E \propto DB \propto x^{-0.75} x^{0.75} \propto x^{0}$), E should be independent of body size (*adapted from* Loeuille and Loreau 2006). Arneberg and Andersen (2003) revealed an erroneous deviation from the EER due to the sampling bias of pitfall trapping when compared to an area-based sampling unit (quadrat). Ordinary least squares regression across 47 communities yielded slopes for body size (via dry weight) verses abundance of 0.18 (pitfall trapping) and -0.64 (quadrat sampling), and tested these slopes against the EER rule expectation of -0.75. The authors rejected the EER for pitfall trapping but not for quadrat sampling, and suggested the explanation was that larger beetles tend to dominate

pitfall trap catch (Andersen 1995). Following Arneberg and Andersen (2003), the present study explicitly tests the relationship between abundance and dry weight (as well as body length) for pitfall trapping and litter extraction.

Trophic Identity

Non-normative observations for numerical dominance obtained by pitfall trapping are further complicated in that the dominance order produced for trophic identity is also counter to prediction. Following observations for foliar-dwelling Insecta (Root and Cappuccino 1992, Siemann et al. 1999), numerical dominance by herbivores is expected over carnivorous species. Contrary to this normative expectation, the summary list produced by Luff (2002) places carnivorous groups as dominate over omnivorous groups primarily composed of seed feeding genera (e.g. *Bembidion* > *Harpalus*, *Pterostichini* > *Harpalini*).

Site selection via species site-specificity

During the planning stage of nontarget studies, candidate field sites must be identified which support a minimum abundance and/or diversity of species to facilitate continuous monitoring. This question is of particular interest in agroecosystems where regular disturbance or land use patterns may result in too little insect biodiversity to conduct sound study. In highly disturbed systems, it may be difficult to infer links between species and novel stressors within the study plot, as detected species may have effectively been vagrant or tourist species (Dufrêne and Legendre 1997). In the present study, site

selection is retrospectively examined using measures of carabid abundance and species site-specificity.

Daytime versus nighttime litter extraction.

Finally, an important consideration for active sampling methods, including litter extraction, is the influence of time-of-sampling. Diel activity of carabid adults has been examined using time-sorted pitfall trapping (Alderweireldt and Desender 1990, Kegel 1990). The influence of sampling time-of-day has not been examined for litter extraction using the near-absolute method described herein. Sampling time-of-day would not be predicted to result in differences for small- to intermediate-bodied carabids, as smaller beetles are not expected to burrow deep enough within field plots to avoid vacuum sampling, and smaller beetle densities are often greater in disturbed habitats (managed grasslands, Blake et al. 1994; urban environments, Magura 2006). Therefore, larger species were predicted to be collected more often at night, when they were assumed to have entered field plots from surrounding non-crop habitats or in-field burrows. If time-of-day litter extraction does not influence species detection, then biomonitoring programs could operate during the most convenient period and without concern of overlooking species active only during diurnal or nocturnal periods. Studies included a time-of-day sampling treatment for litter extraction to examine its effect on species detection.

Objectives

The two main objectives of this study were sampling method comparison and candidate bioindicator identification. Sub-objectives included examining the influence of sampling method on the following measures: (1) sex ratio bias; (2) capture efficiency (species richness, empty sample occurrence, species composition); (3) species dominance (abundance, biomass, and trophic identity); (4) abundance as explained by body length and biomass; and (5) site selection via species site-specificity (empty sample occurrence, species composition). Additionally, sampling time-of-day was examined for litter extraction. Candidate bioindicators were selected by comparing results from both sampling methods and relating them to criteria listed for bioindicator selection.

Materials and Methods

Study Sites

Three general farm types representing corn growing regions and agricultural practices were selected to provide a cross-section of Maryland field corn-systems. Each of the three farm types fall into broad classes describing degrees of habitat fragmentation (Table 1.1). The first farm type, strip cropping, is found near the base of the Piedmont plateau, and includes strip farming with fields surrounded by a majority of wooded edge habitat, typically in a corn – soybean rotation. The second farm type, mixed border, is found on the Eastern Shore, and includes medium sized fields with approximately 50 percent of fields bordering expanses of woods or grass buffer strips (non-crop habitats), and the remaining border with other crops and minimal non-crop habitats. The third farm type,

monocropping, is also found on the Eastern Shore, and is characterized by large, open fields with minimal non-crop habitat edges. Three fields for each farm type (nine sites total) were used in the study. All farm sites utilized no-till practices, common in Maryland. Additionally, reduced tillage practices have been associated with greater carabid species richness than conventional tillage (Menalled et al. 2007, Albajes et al. 2009).

Sampling methods

To examine arthropod diversity and abundance at each site, sampling methods included both pitfall trapping and litter extraction (vacuum sampling and berlese method). At each farm site, a single corn field or strip (study plot) was selected for pitfall trapping. Within a single row at the center of each plot, three unfenced pitfall traps were established with 25 m spacing. Traps were positioned at fixed locations throughout the study, and only opened during each sampling cycle. Each trap consisted of two stacked 355 mL clear plastic cups (TP12; Solo Cup Company, Highland Park, IL) buried with the rim level to the soil surface. The outer cup remained in place to prevent reburial when servicing the trap. The inner cup, containing 100 mL of ethylene glycol preservative, was sheltered with a 23 cm^2 black plastic cover to reduce contamination by rain water and debris. Pitfall trap diameter (9 cm) was expected to be sufficiently wide enough to estimate multiple population parameters (Work et al. 2002). Pitfall trap samples were retrieved at the end of each sampling cycle, filtered to remove preservative and stored in 70% ethanol prior to taxonomic identification.

Litter extraction (vacuum sampling and berlese method) sampling locations within each study plot were chosen by randomly selecting a number of rows (between 5 and 20) to the left or right of the pitfall trap row, and then vacuuming was conducted along a transect aligned parallel to the pitfall trapline. Vacuum samples were spaced at 20 m intervals. Vacuum sampling was conducted using a G-vac leaf blower/vacuum (ES-210 Shred'N'Vac®, Echo Inc., Lake Zurich, IL; maximum air volume: 7.7 m^3 min^{-1} (271 cfm); maximum air velocity 65 m s^{-1} (145 mph)) connected downstream to a collection chamber (Figure 1.1), and rigid vacuum tubing. The collection unit input tubing and sampler head measured 6.3 cm in diameter (31 cm^2 area). Vacuum samples were deposited into mesh sample bags (600 micron mesh, model 11523/12212, Trimaco Inc, Durham, NC), and maximum sample volume was limited to ≈7.6 L (2 gal) by a sample transport bucket contained within the collection chamber. The study plot area sampled (≈0.31 m^2 [480 sq in.]) was defined by six corn plants divided across two rows, including the in-row and row inter-spaces (Figure 1.2). Each vacuum sample consisted of placing the G-vac sampling head ≈2 cm above the soil surface, positioned in the upper left quadrant of the sample area, and sweeping the sample area beginning with the perimeter and finishing with the row inter-space. Sampling was continued until all loose surface matter was collected (≈20 s). Vacuum samples were returned to the laboratory, and arthropods were extracted using Berlese funnels until all sample material was dry (≈3 d). Following berlese extraction, samples were manually sorted to ensure species tolerating xeric environments (e.g. *Amara* spp.) were recorded. All arthropods collected via litter extraction were stored in 70% ethanol prior to taxonomic identification.

Sample timing and crop phenology

Sampling was conducted in field corn during three crop phenological events related to potential toxin exposure: (1) canopy closing during late whorl (V8-V10; mid-June) when early effects of seed treatments may be revealed (Dively et al. in prep.), (2) anthesis (R1-R2; late-July), when fresh pollen containing transgenic toxins enter the epigeal habitat (Chapter 3), and (3) post-harvest (late-September, early-October), when crop residues containing transgenic toxins are deposited on the ground (Zwahlen et al. 2003). Farm sites were grouped into sets of three by geographic proximity, and sampled in random order during each crop event using the following scheme. At the beginning of a sampling cycle, pitfall traps were deployed at each of the nine sites and kept open until all sites had undergone the vacuum sampling process (approximately two weeks). For each site, ten vacuum samples were taken once in 24 hours; five during the day (1200 h – 1600 h), and five at night (2200 h – 0200 h).

Measured Variables and Analyses.

Arthropod identification

Adult carabids were identified to species and sexed based on the presence of external male characters (e.g. expanded protarsi, adhesive pads) or genitalia. Other common arthropod taxa were identified to the family-level or grouped by trophic function. Highly abundant groups (e.g. soil mites) were estimated using grid sub-sampling, and voucher samples were identified to species.

Community representation

The influence of sampling method was examined for the following measures: (1) sex ratio bias; (2) capture efficiency; (3) species dominance (abundance, biomass, and trophic identity); (4) distribution of abundance by body length classes; (5) abundance as explained by body length and biomass; and (6) site selection via species site-specificity.

Male:female

Male:female ratios for each of the three crop events were compared between sampling methods to examine potential gender bias. Ratios were converted to decimal form and analyzed using ANOVA (PROC MIXED; SAS Institute Inc. 2008).

Capture efficiency

Capture efficiency was first examined as the influence of sampling time-of-day for litter extraction only (empty samples and species assemblage). Then, capture efficiency was compared between sampling methods using three measures: (1) the number of species collected (species richness); (2) the percentage of empty samples; and (3) species capture (species assemblage).

The influence of sampling time-of-day on capture efficiency was first examined by comparing the percentage of empty samples between day and night sampling periods. Empty samples were defined as those containing zero carabid adults but containing other arthropods (bycatch). The presence of bycatch served as a positive control to validate each sample. Second, sampling time-of-day was examined with multivariate techniques

addressing capture of carabid adults and bycatch separately (see Multivariate statistical analyses). For sampling method comparison, multivariate analyses enabled separation of carabid species into groups collected most by each method (litter extraction, pitfall trapping), revealing the species assemblages estimated by either sampling method alone. Equal capture by both methods at either specific or generic taxonomic levels would indicate sampling method redundancy; dissimilar assemblages would suggest uneven capture of the carabid community between sampling methods. Beyond examination of sampling method redundancy for species assemblage estimates, the key product here is identification of bioindicator candidates most effectively captured using each sampling method.

As both sampling methods utilize different modes of capture, each is expected to contribute a complementing proportion of the total carabid community detected by both methods combined (species assemblage). To examine the influence of sampling method on species richness estimation, the number of shared species (observed) and the estimated number of species (observed plus unobserved) were calculated. Because the selected sampling methods have different units of measure, it is not appropriate to compare them using shared species estimators based on abundance data (abundance-based estimators). Rather, only incidence-based (presence/absence) species estimators may be used. Therefore, Jaccard's (1901) species similarity coefficient (incidence-based) was calculated using two pooled samples, one for each method. For the same reason, sampling methods were independently analyzed using the Incidence-based Coverage Estimator (ICE) to estimate species richness for observed and unobserved species

(Chazdon et al. 1998, Chao et al. 2000). Coverage estimators emphasize the occurrence of "rare" species (e.g. singletons, doubletons) in estimating species richness (observed plus unobserved) compared to weighting widespread or abundant species that provide little additional information in estimating the richness of an assemblage (Magurran 2004). Diversity statistics were calculated using EstimateS (Colwell 2005) set at 100 randomizations for species richness estimation and 10 individuals as the upper limit for rare species. The resulting output was used to generate species accumulation curves for each method (number of species detected as number of samples increased).

The second measure of capture efficiency included examination of the percentage of empty samples between sampling methods using ANOVA (PROC MIXED; SAS Institute Inc. 2008). As sampling methods had different units of measure, the number of individuals collected per sample were not directly compared. However, to provide basic descriptive statistics, the means of all sub-sampled litter extractions and pitfall traps are presented, where pitfall trap data was standardized as the number of carabid adults per sample, per day (trap days).

Finally, to examine differences in species detected by each method (species capture efficiency), relative differences in abundance were compared between methods over each cropping event in a multivariate analysis (see Multivariate statistical analyses).

Species dominance: abundance, biomass, and trophic identity

To compare the dominance structure described by each sampling method, tabulated species abundances were ranked by total abundance and re-ranked by biomass (sum of individual dry weights). To obtain individual dry weights, each carabid adult was washed in acetone, then washed in water, dried in an oven (Fisher Isotemp® oven, model 230G, Fisher Scientific, Pittsburgh, PA) at 70°C for 48 h, and weighed on a C-33 microbalance (ATI Cahn, Boston, MA; accuracy: ± 0.002 mg, precision: 0.001 mg). Trophic identity was assigned for each species based on the literature (Appendices E and F). Each species was classified as either a carnivore (e.g. primarily animal-based diet, including saprophagy) or omnivore (e.g. animal and vegetal dietary components, *senso lato* granivory). Dominance rankings between methods were compared to normative standards in the pitfall trapping literature for the following: (1) numerical dominance; (2) biomass dominance; and (3) numerical dominance parsed by trophic identity.

Distribution of abundance by body length

To examine body size (via body length) as an explanatory variable distinguishing sampling methods, the median body size for each species (by gender) was calculated for a sub-set of individuals collected, then assigned to each recorded specimen (Appendices G and H). The resulting data was then plotted against abundance to seek natural groupings of body lengths in the study system. In the following section, body size and abundance relationships were formally analyzed.

Abundance versus body length and biomass

To test the hypothesis that sampling methods produce conflicting relationships between carabid abundance and body length or mean individual biomass (MIB), all data were natural log transformed and relationship slopes were compared using the SOLUTION option of PROC GLM (SAS Institute Inc. 2008). Where differences in regression slopes between sampling methods were detected ($\alpha = 0.05$), transformed data were analyzed using linear regression (PROC REG) to model the relationship. Where regression detected a significant relationship for abundance versus MIB ($\alpha = 0.05$), slopes were tested against the EER assumption (slope = -0.75) using a TEST statement (PROC REG; SAS Institute Inc. 2008). Using ordinary least squares regression, it is assumed there is no error variance in the independent variable (see Arneberg and Andersen 2003). In order to avoid violation of this assumption, body length classes of 5 mm were used to group species into class variables; thus eliminating variance in the independent variable (body length), with the trade off of reducing biological resolution.

Site selection via species site-specificity

To retrospectively examine field site selection, three measures were examined: (1) percentage of empty samples (e.g. presence of minimum abundance), (2) species composition (site-specificity), and (3) correlation with environmental attributes.

Minimum abundance was assessed between farm sites based on the percentage of empty samples (did not contain carabid adults but did contain bycatch). Inordinately low catch rates may identify sites that do not support enough abundance or biodiversity to enable

further study. Percentage of empty samples was calculated separately for pitfall trapping, daytime and nighttime litter extraction, at each site during each cropping event. Percentage of empty samples was then compared across study sites using ANOVA (PROC MIXED; SAS Institute Inc. 2008).

To examine the influence of site selection on species composition, a multivariate analysis was conducted with farm study site as an explanatory variable, and individual species as the response variables (see Multivariate statistical analyses). This technique has been utilized for analyzing epigeal community responses in nontarget studies of transgenic crops (French et al. 2004, Dively 2005).

Using pitfall trapping, environmental attributes have been shown to influence carabid presence and abundance (Appendix A). Litter extraction has been underexamined as a technique for studying habitat selection by carabid adults. To examine if environmental attributes influenced species composition in addition to farm site type, the following variables were point sampled once during each daytime vacuum sampling trip: soil moisture (SoilM), weed cover (Weeds), and thatch depth (Thatch). Soil moisture was estimated within the sample plot using a probe (HB-2 Kelway soil pH and moisture meter, Kel Instruments, Wyckoff, NJ; moisture accuracy: ± 10%). Percent weed cover and thatch depth (cm) were both estimated for a random ≈100 m^2 area within each study plot.

Multivariate statistical analyses

Multivariate analyses were conducted to examine the influence of the following explanatory variables on species composition: daytime versus nighttime litter extraction by cropping event, sampling method by cropping event (species capture efficiency), farm site selection, and environmental attributes (soil moisture, weed cover, thatch depth). Multivariate analyses were performed using redundancy analysis (RDA), a constrained form of principal component analysis (PCA), which seeks the underlying structure of the species data and then relates that structure to the explanatory variables (constraints). All RDA were performed using CANOCO version 4.54 (Biometris, Plant Research International, Wageningen, The Netherlands). Monte Carlo permutation tests were used to examine the significance of species composition patterns as they related to explanatory variables (ter Braak and Šmilauer 2002). The significance level was determined by the proportion of F values greater than or equal to the F value based on the original data set. Permutations were conducted within split-plots (e.g. crop-event-by-method), and blocked by covariates (e.g. farm site) where appropriate. With the exception of capture efficiency, analyses for litter extraction included data only from sites on the eastern shore of Maryland, as replication at these sites was fully balanced across each cropping event after means of sub-samples were taken. In capture efficiency analyses, all litter extraction data was used since sub-sample means across all Maryland sites produced a balanced design. In all but one case (whole community analysis), carabid species and bycatch were examined separately to reduce clutter on biplots. Prior to analysis, all raw abundance data (mean of subsamples per site by method for each cropping event) were natural log transformed using the formula:

$$y' = \ln(a*y + b)$$

where y' = transformed species abundance, y = raw species abundance, a = 10 and b = 1. As sampling methods produce species abundance measures with different units, all analyses included both centering and standardization of species data. Additionally, scaling was focused on inter-sample distances, and species scores were divided by the standard deviation for each species. From the combination of these two options, a correlation biplot is obtained and the length of a species' vector on each biplot is then a measure of fit (R) with the ordination diagram (ter Braak and Šmilauer 2002). Finally, before RDA, each species data set was examined using detrended correspondence analysis to confirm linearity of data (Lepš and Šmilauer 2003). In each case, the longest of all gradients never exceeded 3.0, suggesting linear ordination methods (e.g. PCA, RDA) were appropriate.

Results

Arthropod identification

Over the course of study, pitfall trapping yielded 368 carabid adults from 78 traps, and litter extraction yielded 1925 adults from 250 samples. Carabid species and number collected during each cropping event are presented in Table 1.3; the mean number of carabid adults collected (± SE) by each sampling method is presented in Table 1.4. The bycatch totals were 12,817 individuals for pitfall trapping, and 159,671 individuals for

litter extraction. Bycatch taxonomic groupings and means (± SE) of sub-samples for both sampling methods are presented in Table 1.4.

Male:female

Male to female ratios between methods were significantly different ($F = 12.40$; df = 1, 4; $P = 0.024$) with pitfall trapping exhibiting a 1:1.6 (0.64 ± 0.06) and vacuum sampling exhibiting 1:1.1 (0.93 ± 0.06) (Appendix I).

Capture efficiency

A total of 49 carabid species were detected using both sampling methods (Table 1.3). Pitfall trapping captured 31 species and did not detect 18 species captured by litter extraction. Litter extraction captured 43 species and did not detect 6 species captured by pitfall trapping. Both methods shared detection of 25 species producing a Jaccard (1901) species similarity coefficient (incidence-based similarity measure) of 0.51. Using the Incidence-based Coverage Estimator (ICE), species richness (including unobserved species) was estimated at 36 species for pitfall trapping, and 63 species for litter extraction (Figure 1.3).

Over all cropping events, there was no difference between daytime or nighttime litter extractions for the percentage of empty samples ($F = 0.41$; df = 2, 42; $P = 0.663$) (Table 1.2), or species composition (carabid adults: $F = 1.19$; $P = 0.102$; bycatch: $F = 1.15$; $P = 0.192$) (Table 1.5). Based on these findings, sampling time-of-day was removed from all other analyses for carabid adults in order to increase sub-sampling for litter extraction.

The percentage of empty samples did not differ between methods over the course of study ($F = 0.21$; df = 1, 24; $P = 0.651$) (Table 1.2), where both methods produced approximately 23% empty samples.

Capture efficiency of individual species between methods was significantly different over cropping events (Table 1.5) for carabid adults ($F = 2.03$; $P = 0.004$) (Figure 1.4) and all taxa combined ($F = 5.93$; $P = 0.004$) (Figure 1.5). Capture efficiency for each taxa is indicated on each biplot, where the length of the taxa vector reflects the correlation of each taxa to the explanatory variables, and is equivalent to the measure of fit (R). Clear differences in capture efficiency between sampling methods was evident at the species- and genus-levels for carabid adults (Figure 1.4) and at the family-level for the epigeal community (Figure 1.5).

For highly abundant soil mite taxa (Table 1.4), randomly selected voucher samples identified the following species in Maryland field corn: *Ceratozetes enodis* (Ewing) (Ceratozetidae), *Exoribatula biundata* Jacot (Scheloribatidae), *Galumna jacoti* Marshall et al. (Galumnidae), *Pergalumna corrugis* (Jacot) (Galumnidae), *Scheloribates* sp. (Scheloribatidae), and *Zygoribatula rostrata* Jacot (Oribatulidae).

Species dominance: abundance, biomass, and trophic identity

Ranked abundance and biomass of carabid adults are presented for pitfall trapping (Table 1.6) and litter extraction (Table 1.7). Readily identifiable patterns differed based on sampling method. For pitfall trapping: (1) the top five most abundant species were (in

order of decreasing activity density) *Harpalus pensylvanicus*, *Stenolophus ochropezus*, *Amara aenea*, *Chlaenius tricolor*, and *Scarites subterraneus*; (2) six of the top ten most abundant species were classified as carnivores; (3) when ranked by abundance, individual biomass and median species body length showed no pattern; (4) when ranked by individual biomass or median species body length, a general pattern revealed dominance by larger (>10 mm length) (and more massive) species; and (5) the single most abundant species (*H. pensylvanicus*) was also the most biomassive. For litter extraction: (1) the top five most abundant species were (in order of decreasing density) *Stenolophus conjunctus*, *Amara familiaris*, *Amara aenea*, *Acupalpus partiarius*, and *Stenolophus ochropezus*; (2) all ten most abundant species were classified as omnivores; (3) when ranked by abundance, individual biomass and median species body length revealed dominance by smaller (and less massive) species; (4) when ranked by biomass intermediate body sized species (5-10 mm length) dominated; and (5) the most abundant species (*S. conjunctus*) was less biomassive than subdominant species in the genus *Amara* (i.e. *A. aenea*, *A. familiaris*).

Focusing on carnivores, the top five most abundant species collected using pitfall trapping were (in order of decreasing activity density): *Chlaenius tricolor*, *Scarites subterraneus*, *Scarites quadriceps*, *Tetracha virginica* (formerly *Megacephala virginica*), and *Poecilus chalcites* (Table 1.6). The top five most abundant carnivores collected using litter extraction were: (in order of decreasing density) *Elaphropus xanthopus*, *Agonum punctiforme*, *Elaphropus anceps*, *Calathus opaculus*, and *Notiophilus novemstriatus* (Table 1.7).

Distribution of abundance by body length

Visual examination of abundance distributions by body length revealed no discernible pattern for pitfall trapping data (Figure 1.6), and a right-skewed (positive skew) distribution for litter extraction data (Figure 1.7). Examining the distribution of carabid body lengths using both sampling methods, gaps in abundance appeared at the 5, 7, 10, and 14 mm marks. Following these results, species abundances were grouped into 5 mm body length classes (to five decimal places) and analyzed using linear regression, results below.

Abundance versus body length and biomass

Regression slopes between sampling methods for abundance against body length class were significantly different ($F = 11.93$; df = 2, 7; $P = 0.006$), where pitfall trapping showed no relationship ($P = 0.975$; $R^2 = 0.0004$) (Figure 1.8), and a negative relationship was detected for litter extraction ($P = 0.012$; $R^2 = 0.829$; RMSE = 1.420; ln(density) = ln(10.765) + -2.983*ln(body length class)) (Figure 1.9). Correspondingly, regression slopes between methods for abundance against mean individual body mass (MIB) were significantly different ($F = 11.19$; df = 2, 7; $P = 0.007$), where pitfall trapping data showed no relationship ($P = 0.952$; $R^2 = 0.001$) (Figure 1.10), and a negative relationship was detected for litter extraction ($P = 0.013$; $R^2 = 0.817$; RMSE = 1.470); ln(density) = ln(7.448) + -1.293*ln(MIB)) (Figure 1.11). In a test of the energetic equivalence rule (EER) assumption that the slope of abundance versus MIB should be approximately -0.75 was not rejected for litter extraction data ($b = -1.293$; $F = 3.15$; df = 1, 4; $P = 0.151$). The

finding of no relationship between abundance and MIB for pitfall trapping resulted in rejection of the EER.

Site selection via species site-specificity

Examining sampling methods jointly, the percentage of empty samples was significantly different across farm sites ($F = 6.73$; df = 8, 24; $P = 0.0001$), although there was no trend based on farm type (Tables 1.2). However, the site with the single highest percentage of empty samples was the largest plot sampled (CHI), and was bordered by large areas of monocropped habitat. Species composition was significantly different for both carabid adults and bycatch between sites for both sampling methods (Table 1.5).

For biplots of species correlations to farm site for pitfall trapping (Figure 1.12) and litter extraction (Figure 1.13), short vectors reflect species that were found more evenly across sites, and long vectors reflect species that were found exclusively at each site (site-specificity). Of the few taxa with short vectors, most were captured in very low numbers. Therefore, no single species or genus exhibited high fidelity, characterized by even distribution and high abundance. However, normally common species (via pitfall trapping literature) were only found in great abundance at specific sites using either pitfall trapping or litter extraction.

Sites exhibiting the lowest diversity of carabid species (CHI, WYE) were generally clustered together using both sampling methods (Figures 1.12 and 1.13). For pitfall trapping, the majority of omnivorous carabid species detected were collected at the

HOPE site; the remaining sites were more clustered due to shared detection of the remaining species collected (Figure 1.12). For litter extraction, sites GER and HOPE were identified for their high carabid diversity, where both supported a mixture of carnivorous and omnivorous species (Figure 1.13).

Species composition in relation to environmental attributes was variable between methods and taxa groupings. Significant differences in species composition were only detected for carabids using pitfall trapping ($F = 1.35$; $P = 0.022$), and bycatch using litter extraction ($F = 1.64$; $P = 0.030$) (Table 1.5). For pitfall trapped carabids, many species were negatively correlated with environmental attributes (Figure 1.16). For litter extracted bycatch, taxa were generally correlated with thatch depth and weed cover, rather than soil moisture (Figure 1.17).

Discussion

Relative to the normative expectations of greater abundance for smaller-bodied species and dominance by omnivorous species, pitfall trapping and litter extraction methods produced very different descriptions of the carabid populations inhabiting Maryland field corn. Although pitfall traps were deployed for relatively long intervals (14 d) compared to point-sampled litter extractions, litter extractions produced larger estimates for species richness and population density. Differences in capture efficiency between methods may be explained in part by body size bias of pitfall trapping, which failed to detect several small- and intermediate-sized carabid species. While pitfall trapping is required to

efficiently monitor the presence of larger bodied species, litter extraction enables estimates of all expected body sizes (Lang et al. 1997) and in proportion to their expected densities and trophic affiliations. Vacuum-based litter extraction enables faster sample extraction for area-based arthropod densities than previous methods, and is suggested for use in programs monitoring ground beetle populations.

Body size factors

The sampling methods tested here produced carabid species compositions that differed in their relationships of abundance versus body length and mean individual biomass (MIB) (Figures 1.8-1.11). The relationship between population density and individual biomass (and body size) is expected to be negative (Currie 1993). The zero slopes for population-level activity density (pitfall trapping) plotted against body length (Figure 1.8) or individual biomass (Figure 1.10) suggest, along with previous studies, that pitfall trapping is unreliable for obtaining true population-level parameter estimates. The results here support the findings of Arneberg and Andersen (2003), in which the energetic equivalence rule (EER) was rejected for pitfall trapping data but not for quadrat data. While the resulting slopes of density versus body length and biomass for litter extraction were not rejected based on EER assumptions, slope values were slightly more negative than the EER assumes (-0.75). This finding suggests that small body sizes are more common, and/or larger body sizes are more uncommon in Maryland field corn systems than would be expected in unmanaged systems. This finding also supports previous studies in managed grassland systems where larger body sizes were less common due to disturbance (Blake et al. 1994). For carabid adults in Maryland field corn, smaller body

sizes (>0-5 mm length) are the most abundant, but intermediate body sizes (5-10 mm) (e.g. *Amara* spp.) may explain the majority of biomass consumption in field corn systems. This is likely explained by food availability in the cropping environment, and a larger diet breadth, as *Amara* spp. exploit weed seed of various sizes (Honěk et al. 2007) as well as microinvertebrates (Larochelle 1990), whereas smaller species may be restricted to smaller seeds and prey.

Using litter extraction, the present work found small- and intermediate-body sized species to be an order of magnitude more abundant than large-bodied species (Table 1.7). Greater carabid density for small-bodied species using a relatively small sampling area (≈0.3 m^2), suggests a more even distribution of single species within sites and therefore more homogenous study plots for field-scale experiments. Carabid body size is of particular interest relative to study plot size selection in nontarget studies examining transgenic crops (Prasifka et al. 2005). Some larger carabid species operate at farm-size scales (Baars 1979) via cursorial dispersion (Best et al. 1981), and flight capable species of all body sizes also regularly disperse (den Doer 1969). Use of pitfall trapping in nontarget monitoring may require knowledge of species mode of dispersion, habitat fidelity, and habitat scale in order to select study plot sizes that reduce focal species exposure to multiple experimental plots. If smaller species operate on a smaller scale within the cropping environment and do not regularly flight disperse, then confounding exposure to multiple experimental plots may be reduced, resulting in increased study sensitivity. In managed or disturbed systems, smaller carabid species are typically retained whereas larger species diminish (Blake et al. 1994, Braun et al. 2004). In cropping systems,

regular disturbance may reduce large-bodied species densities to levels that prohibit adequate assessment of population change when a novel stressor is introduced. Under disturbed circumstances, smaller species may then comprise the majority of the community from which response to environmental stressors can be measured.

An important confounding factor in transgenic crop monitoring studies following regulatory approval is the presence of broad-spectrum insecticidal seed coatings which are prepackaged in addition to transgenic insecticidal traits. In the case of Bt endotoxins, detection of potentially subtle effects are most likely precluded by the overwhelming negative effect of seed treatments on some but not all taxa in the target group. For example, large-bodied carnivorous species in the genus *Scarites* are likely disproportionally exposed to toxic seed treatments (Mullin et al. 2005) due to burrowing behaviors (G.P. Dively, unpubl. data; M.D. Lepping, pers. obs.). Confounded exposure to prepackaged seed treatments may reduce the utility of burrowing species as bioindicators of transgenic toxin exposure. Thus, examination of smaller-bodied species or species that exhibit reduced burrowing once adult eclosion occurs may provide the opportunity to limit confounding effects in field studies.

It is assumed that taxa detected within a study plot are present to utilize resources in that plot. While linking predator-prey relationships within field sites was beyond the scope of the present study, the bycatch estimated in litter extractions provides useful information on the availability of potential food resources. Food availability may partly explain the higher densities of smaller carabids compared to larger ones. Litter extraction revealed

high abundances of small-bodied taxa, especially soil mites, collembola, and fungivorous Coleoptera (Table 1.4). The egg and immature stages of these groups are known prey of carabid beetles (Pollet and Desender 1989, Larochelle 1990) and may provide consistent food resources that could increase retention within study plots. Large-bodied species that forage on large spatial scales may require food resources in crop and non-crop habitats, thereby reducing site fidelity (abundance plus even distribution). The relationship between carabid body size and spatial scale of resource utilization should be examined to substantiate the selection of smaller species as appropriate bioindicators of in-field disturbances.

Species richness and sex ratio

Neither method captured all 49 species detected by the combination of sampling methods (Table 1.3). In terms of species richness estimation, richness was underestimated by a minimum of 37% for pitfall trapping, and by a minimum of 12% for vacuum sampling. Examining species abundance curves to estimate total richness (observed plus unobserved) (Figure 1.3), it is evident that the uncommon species detected by litter extraction enable estimation of a total richness (63 species) that better approximates the actual number detected using both methods (49 species) than the underestimation predicted by pitfall trapping alone (36 species). These findings suggest that litter extraction increases species detection and may replace pitfall trapping for species richness estimation when only one sampling method is feasible. However, litter extraction coupled with pitfall trapping provides the best estimation of species richness. Over long periods, the combination of sampling methods with different modes of capture

invariably increases the total number of species detected (pitfall and light trapping; Yahiro and Yano 1997). However, for short term studies that combine pitfall trapping with labor intensive litter extraction methods, additional species are not necessarily garnered (soil drenching: Andersen 1995, hand searching: Lin et al. 2005), as these methods may ineffectively extract small-bodied species. The present work reveals that vacuum-based litter extraction provides a solution for capturing a majority of carabid species richness over a short period of time.

The ratio of male to female capture was lower for pitfall trapping (1:1.6) compared to litter extraction (1:1.1) (Appendix I). Sex ratios add dimension to risk assessment as environmental stress on females may result in direct effects on species reproduction. Due to the differences in unit of measure and species composition it is difficult to determine if either sampling method is gender biased. Neither method is known to have an intrinsic bias for either gender.

Site selection via species site-specificity

When identifying agricultural sites for bioindicator monitoring, it is imperative to select sites with the highest in-field habitation or likelihood of species re-establishment each year. Within the scope of a single year study, sites that supported consistently detectable abundances may be chosen for future field-scale studies. In the present study, sites varied in carabid presence and composition. Although data did not suggest that field size was a strong predictor of carabid abundance, specific farm sites revealed insightful patterns. For example, the largest farm site examined (CHI) exhibited the highest percentage of empty

samples across both sampling methods. This site was bordered by minimal non-crop habitat including ditches and paved roads separating it from additional large, monocropped fields on all sides. The paucity of abundance and diversity at this site, in comparison to highly diverse sites (e.g. GER) suggest the CHI site may not support field-scale nontarget studies. Conversely, the high diversity at the GER site was partly due to the large border of non-crop buffer areas (conservation strips) between fields and wooded habitats. Proximity to non-crop areas introduces many species that may not exhibit regularity or specificity to agricultural sites; hence proximity would be expected to introduce undesirable variation in monitoring studies. For example, at least one of the captured species (*Calosoma scrutator*) could be classified as a vagrant or tourist species. Such species are not expected to depend upon crop resources, thus may be considered noise in estimation of diversity associated with field corn systems.

None of the species collected are considered uncommon or rare. The dominance of common species at multiple sites suggests that eurytopic (habitat generalist) species may be the most appropriate when the bioindicator criteria of site selectivity and fidelity are considered. It should be noted that abundance by itself is not an ecologically important issue in risk assessment, as both rare and common species are of interest. Determination of appropriate study sites and sampling methods is rooted in the practical need to obtain enough data to produce a robust analysis.

The identification of appropriate study sites may then include selecting sites that: (1) support an abundance of study organisms; and (2) enable definition of large test plot sizes

and associated buffer areas that may reduce exposure of study organisms to multiple field plot treatments (i.e. cross-contamination; Chapter 3). Large test plots would be feasible at farm sites such as GER and HOPE, which were in the intermediate class (≈7 ha) of study plot sizes examined and supported some of the highest carabid densities and taxa richness (Figures 1.12 and 1.13).

Candidate bioindicators

Based on criteria for ideal bioindicators in field studies, candidate species should be accessible for monitoring and representative of their taxonomic or functional group. The sampling methods tested here produced opposing descriptions of species dominance for abundance and biomass, and therefore jointly identified three distinct groups based on body size. Since it is difficult to defend bioindicator selection for species based on semi-quantitative techniques (e.g. pitfall trapping) when their findings do not agree with quantitative measures (e.g. quadrat sampling, litter extraction) (Lang 2000), litter extraction data is weighted more heavily in consideration of bioindicator candidacy. Based on sampling feasibility, population dominance, and trophic identity, the following groups are suggested for monitoring in field corn as candidate bioindicators of pesticide exposure.

The first group is composed of numerically dominant small-bodied carabid (>0-5 mm length) carnivores (Bembidiini: e.g. *Elaphropus*) and omnivores (Harpalini: e.g. *Stenolophus*). The second group includes intermediate sized (5-10 mm length) omnivorous *Amara* spp. (Zabrini), which accounted for the most biomass and numerical

subdominance. The third group includes larger bodied (10+ mm length) carnivores (e.g. Pterostichini, Scaritini) and omnivores (Harpalini: e.g. *Harpalus*). Species composition shifts throughout the year provide the opportunity to focus on specific taxa. Species composition also varies widely depending on geographic location. Therefore, tribe-level representation may be adequate depending on the environmental stressor(s) and study questions.

From canopy close through anthesis, species in the genera *Scarites* (Scaritini) and *Elaphropus* (Bembidiini) represent large- and small-bodied carnivores, respectively. *Scarites* spp. and other burrowing predators may be appropriate indicators of exposure to broad-spectrum pesticide treatments applied as seed coatings (Leslie et al. 2009, Dively et al. in prep.). Exposure to transgenic toxins is expected to occur through facultative pollen consumption (active or passive) (Romeis et al. 2009). For egg predators (e.g. small bodied adults, larvae of large bodied adults), prey-switching to corn pollen could potentially occur during anthesis, as corn pollen grains are relatively large and may mimic egg prey. *Elaphropus xanthopus* (Dejean) (Chapter 2) and similar species may function as egg predators in the cropping environment. In laboratory studies, the eggs of a soil mite (*Pergalumna corrugis*) were identified as a readily consumed prey item for *E. xanthopus* adults (Chapter 2). Several soil mite species, including *P. corrugis*, were highly abundant in field corn plots (Table 1.4), where their egg stages may support abundant, small bodied carnivores including *Elaphropus* spp. While soil mite populations may exceed those of similar sized potential prey species, corn pollen grain availability would be expected to exceed soil mite egg availability during anthesis. Anthesis may then

present the opportunity for direct exposure to transgenic plant tissue by small bodied carabids.

Species in the genus *Amara* are present throughout the year but are most abundant in the early season when weed stands or cover crops provide seed resources. However, *Amara* spp. exhibit broad abiotic tolerances and inhabit highly disturbed tilled fields, suggesting sensitivities to environmental stressors that may be too low to enable bioindication of subtle effects.

Highly abundant genera in the tribe Harpalini (*Stenolophus*, *Acupalpus*, *Anisodactylus*, *Harpalus*) dominated throughout the year and peaked late season, providing the opportunity to detect lag effects within the year of study. Although underrepresented in the present study, members of the tribe Pterostichini (e.g. *Calathus*, *Poecilus*, *Pterostichus*) and specialist feeders of snails and slugs (tribes Cychrini and Licinini) would comprise desirable assemblages in no-till field corn systems. In agreement with Lopez et al. (2005) for pitfall trapping-based studies, the high numerical sub-dominance and ubiquity of *Harpalus pensylvanicus* suggest this species is an appropriate large-bodied species for consideration.

Sampling method utility

Pitfall trapping remains the most cost effective solution for monitoring the activity of epigeal taxa (Sunderland et al. 1995). However, pitfall trapping as a primary sampling method is selected with an understanding of the associated biases of the resulting catch.

The outcome is often a low resolution description of the community under study. Lövei and Sunderland (1996) summarized, “pitfall traps should probably not be used to study community patterns such as relative species composition or diversity.” The present results coupled with many studies revealing pitfall trapping bias suggest the pitfall trap is at most a tool for narrow taxonomic description of epigeal fauna. In agreement with Lang (2000), the relationship between pitfall trap catch and actual population density is weak. Therefore pitfall trapping results and their interpretations must remain highly conditional. On the topic of estimating carabid beetle densities, Ekbom et al. (1992) noted, “accurate density estimates of predators is a problem no one has yet solved.” While obtaining accurate density estimates is difficult, coupling accuracy with feasible sampling methodology is an even greater challenge. The litter extraction method described herein offers a high-throughput option for producing density estimates of hard-bodied arthropods. For each measure examined, this method produced consistent and predictable results. Litter extraction estimates for carabid adults fit normative expectations for distribution of body size, biomass, and trophic affiliation. The addition of density estimation via litter extraction may also enable direct comparisons within and between studies. Furthermore, quadrat method techniques have a defined unit of measure that may be scaled relative to study plot size or the scale over which focal taxa operate. For small-bodied carabid predators, quadrat methods enable simultaneous monitoring of predator and prey, which may provide mechanistic explanations of species response for cases where trophic relationships are influenced by environmental stressors. Additionally, the percentage of empty samples, a measure of return on effort, was the same between methods, suggesting the heterogeneous distribution of carabids as a group does not

greatly impair point sampling using litter extraction. High-throughput litter extraction may serve as an important tool for sampling abundant species, and may complement and possibly replace pitfall trapping for species richness estimation when short sampling periods are employed. Finally, this work has enabled identification of potential bioindicator species based on multiple criteria. While satisfaction of comprehensive criteria is difficult, small-bodied omnivores and carnivores are suggested for future study based here on species density over each cropping event, which supports the potential for detecting exposure to environment stressors at the population level.

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Table 1.1. Study sites in Maryland, ordered northernmost to southernmost within each farm type.

Region	County	Site	Site code[a]	Plot size (ha)	Farm type
Piedmont Base	Howard	CMREC Clarksville	CLK	1.8	strip cropping
	Anne Arundel	Davidsonville	COV	0.7	
	Prince George's	USDA BARC	PON	1.3	
Atlantic Coastal Plain	Queen Anne's	Sudlersville	GER	6.4	mixed border
	Queen Anne's	Wye Island	WYE	5.1	
	Talbot	Hope	HOPE	7.5	
	Queen Anne's	Ewingville	CHI	40	mono cropping
	Queen Anne's	Rolphs	COH	20	
	Talbot	Ivytown	SCH	23	

[a] Site abbreviations (Site Code) correspond to site codes depicted in redundancy analysis biplots.

Table 1.2. Frequency table of samples from Maryland cornfields that did not contain carabid adults[a] .

Type	Site Code	Cropping Event[b]									Site Summary			
		Canopy Close			Pollen Shed			Post Harvest						
		Pitfall	Litter Extract.		Pitfall	Litter Extract.		Pitfall	Litter Extract.		empty samples	total samples	% empty	ANOVA (% ± SE)[c]
			Day	Night		Day	Night		Day	Night				
strip cropping	CLK	0 / 3	3 / 5	.	1 / 3	1 / 5	2 / 5	3 / 3	0 / 5	1 / 5	11	34	32	39 ± 6ab
	COV	1 / 3	0 / 5	.	1 / 3	1 / 5	2 / 5	0 / 3	0 / 5	1 / 5	6	34	18	18 ± 6bc
	PON	1 / 3	0 / 5	.	0 / 3	0 / 5	0 / 5	2 / 3	1 / 5	2 / 5	6	34	18	22 ± 6bc
mixed border	GER	1 / 3	0 / 5	0 / 5	0 / 2	3 / 5	2 / 5	1 / 3	2 / 5	0 / 5	9	38	24	23 ± 5bc
	WYE	2 / 3	4 / 5	4 / 5	0 / 3	2 / 4	0 / 4	0 / 3	0 / 5	1 / 5	13	37	35	30 ± 5abc
	HOPE	0 / 3	0 / 5	0 / 5	0 / 3	1 / 2	0 / 5	1 / 2	0 / 5	0 / 5	2	35	6	13 ± 5bc
mono cropping	CHI	2 / 3	1 / 5	1 / 5	1 / 3	5 / 5	4 / 5	1 / 3	2 / 5	3 / 5	20	39	51	49 ± 5a
	COH	1 / 3	0 / 5	1 / 5	0 / 3	0 / 5	1 / 5	0 / 2	0 / 5	0 / 5	3	38	8	9 ± 5c
	SCH	0 / 3	0 / 5	0 / 5	0 / 3	2 / 5	2 / 5	0 / 3	0 / 5	0 / 5	4	39	10	7 ± 5c
Crop Event Summary														
empty samples		8	8	6	3	15	13	8	5	8				
total samples		27	45	30	26	41	44	25	45	45				
% empty		30	18	20	12	37	30	32	11	18				
Method Summary														
empty samples		19	28	27										
total samples		78	131	119										
% empty		24	21	23										
ANOVA (% ± SE)[c]		24 ± 3a	22 ± 2a											

[a] Empty samples are followed by the number of samples retrieved (replication).

[b] Sampling methods employed during each crop event included pitfall trapping (Pitfall) and litter extraction (Litter Extract.); litter extraction was conducted through vacuum sampling and berlese method.

[c] Analysis of variance (ANOVA) estimates for percentage of empty samples (%) followed by the same letter within column (Site) or within row (Method) were not significantly different ($P > 0.05$).

Table 1.3. Number collected, species richness, and taxa code abbreviations for carabid adults captured using two sampling methods in Maryland field corn[a].

Taxa Code[b]	Species	Pitfall Trapping					Litter Extraction (Vacuum and Berlese)				
		Canopy	Anthesis	Harvest	Total	% Total	Canopy	Anthesis	Harvest	Total	% Total
Acr	*Acupalpus partiarius* (Say)	.	5	2	7	1.9	38	73	15	126	6.6
Acu	*A. pauperculus* Dej.	.	.	.	.	.	11	4	24	39	2.0
Ago	*Agonum octopunctatum* (F.)	1	1	.	2	0.5	5	.	1	6	0.3
Agp	*A. punctiforme* (Say)	2	.	.	2	0.5	4	2	13	19	1.0
Ame	*Amara aenea* (DeG.)	31	1	1	33	9.0	210	30	49	289	15.0
Amg	*A. angustata* (Say)	.	.	.	.	.	.	1	1	2	0.1
Amt	*A. anthobia* Vil. & Vil.	.	.	.	.	.	3	.	.	3	0.2
Amc	*A. cupreolata* Putz.	.	.	.	.	.	.	.	1	1	<0.1
Amf	*A. familiaris* (Duft.)	13	1	.	14	3.8	255	23	99	377	19.6
Ami	*A. impuncticollis* (Say)	3	.	.	3	0.8	6	7	4	17	0.9
Ams	*Amphasia sericea* (Harris)	.	.	.	.	.	2	.	.	2	0.1
Anc	*Anisodactylus caenus* (Say)	.	.	.	.	.	2	.	.	2	0.1
Anr	*A. rusticus* (Say)	1	2	.	3	0.8	2	8	11	21	1.1
Ans	*A. sanctaecrucis* (F.)	1	4	.	5	1.4	2	2	4	8	0.4
Ban	*Badister notatus* Hald.	.	.	.	.	.	.	.	1	1	<0.1
Bea	*Bembidion affine* Say	.	.	.	.	.	3	.	.	3	0.2
Ber	*B. rapidum* (LeC.)	.	.	.	.	.	1	.	.	1	<0.1
Brr	*Bradycellus rupestris* (Say)	.	5	.	5	1.4	24	5	38	67	3.5
Brt	*B. tantillus* (Dej.)	.	2	.	2	0.5	1	8	11	20	1.0
Cao	*Calathus opaculus* LeC.	1	.	1	2	0.5	3	1	4	8	0.4
Cas	*Calosoma scrutator* (F.)	.	.	.	.	.	.	1	.	1	<0.1
Ctr	*Chlaenius tricolor* Dej.	2	18	3	23	6.3	.	1	.	1	<0.1
Cip	*Cicindela punctulata* Oliv.	.	2	2	4	1.1	.	.	.	.	.
Clb	*Clivina bipustulata* (F.)	2	.	.	2	0.5	.	.	.	.	.
Die	*Dicaelus elongatus* Bon.	2	1	.	3	0.8	.	.	.	.	.
Dyg	*Dyschiriodes globulosus* (Say)	.	.	.	.	.	.	1	.	1	<0.1
Ela	*Elaphropus anceps* (LeC.)	7	1	.	8	2.2	4	5	4	13	0.7
Elx	*E. xanthopus* (Dej.)	8	.	.	8	2.2	19	.	3	22	1.1
Haa	*Harpalus affinis* (Schr.)	.	.	.	.	.	.	1	.	1	<0.1
Hal	*H. longicollis* LeC.	.	2	.	2	0.5	.	.	.	.	.
Hap	*H. pensylvanicus* (DeG.)	.	39	92	131	35.6	.	8	6	14	0.7
Has	*H. somnulentus* Dej.	.	.	.	.	.	.	16	50	66	3.4
Non	*Notiophilus novemstriatus* LeC.	.	.	1	1	0.3	2	2	3	7	0.4
Pas	*Paratachys sagax* (Csy.)	.	.	.	.	.	.	.	1	1	<0.1
Pal	*Patrobus longicornis* (Say)	.	.	1	1	0.3	.	.	1	1	<0.1
Poc	*Poecilus chalcites* (Say)	7	3	.	10	2.7	1	1	2	4	0.2
Pol	*P. lucublandus* (Say)	.	.	.	.	.	.	.	2	2	0.1
Poa	*Polyderis laevis* (Say)	.	.	1	1	0.3	4	.	2	6	0.3
Pta	*Pterostichus atratus* (Newm.)	.	1	.	1	0.3	.	.	1	1	<0.1
Scq	*Scarites quadriceps* Chd.	14	2	.	16	4.4	.	1	.	1	<0.1
Scs	*S. subterraneus* F.	7	11	.	18	4.9	1	.	.	1	<0.1
Seo	*Selenophorus opalinus* (LeC.)	.	1	.	1	0.3	.	.	.	.	.
Sep	*S. pedicularius* Dej.	.	.	.	.	.	.	1	.	1	<0.1
Stc	*Stenolophus conjunctus* (Say)	.	2	2	4	1.1	83	76	441	600	31.2
Sto	*S. ochropezus* (Say)	4	41	.	45	12.2	54	15	4	73	3.8
Str	*S. rotundatus* LeC.	.	.	.	.	.	2	52	7	61	3.2
Tev	*Tetracha virginica* (L.)	.	10	.	10	2.7	.	.	.	.	.
Trq	*Trechus quadristriatus* (Schr.)	.	.	.	.	.	.	.	4	4	0.2
Trf	*Trichotichnus fulgens* (Csiki)	.	.	1	1	0.3	3	10	18	31	1.6
	Total Collected	106	155	107	368		745	355	825	1925	
	Species Richness	17	22	11	31 nominal		27	27	31	43 nominal	

[a] Cropping event abbreviations, Canopy: canopy close, Anthesis: pollen shed, Harvest: post harvest.

[b] Species abbreviations (Taxa Code) correspond to taxa codes depicted in redundancy analysis biplots.

Table 1.4. Mean (± SE) number of epigeal taxa collected using pitfall trapping and litter extraction in Maryland field corn.

Taxonomic Group	Taxa Code[a]	Common Name	Families	Pitfall Trapping[b]		Litter Extraction[c]	
				n[d]	Mean ± SE	n[d]	Mean ± SE
Acari	smites	soil mites	Ceratozetidae, Galumnidae, Oribatulidae, Scheloribatidae	26	2.00 ± 0.59	27	531.09 ± 124.34
	pmites	predaceous mites	–	13	0.25 ± 0.12	24	27.32 ± 15.31
Araneae	aranea	spiders	–	26	0.73 ± 0.11	27	6.22 ± 1.33
Chilopoda	chilop	centipededs	–	23	0.56 ± 0.18	21	2.42 ± 0.35
Diplopoda	diplop	millipedes	–	17	0.24 ± 0.07	19	4.58 ± 1.14
Collembola	collem	springtails	–	26	5.52 ± 0.95	26	44.77 ± 13.99
Orthoptera	grylli	field crickets	Gryllidae	24	2.86 ± 0.85	17	1.72 ± 0.20
Coleoptera	canthl	soldier beetle larvae	Cantharidae	9	1.38 ± 0.72	13	2.39 ± 0.43
	caraba	ground beetle adults	Carabidae	26	0.42 ± 0.04	27	8.40 ± 1.60
	carabl	ground beetle larvae	"	6	0.07 ± 0.01	13	2.12 ± 0.45
	coccia	lady beetle adults	Coccinellidae	2	0.11 ± 0.06	12	1.31 ± 0.18
	coccil	lady beetle larvae	"	6	0.09 ± 0.02	8	1.06 ± 0.06
	coleol	miscellaneous larvae	–	16	0.16 ± 0.04	26	6.91 ± 1.35
	elatea	click beetle adults	Elateridae	14	0.20 ± 0.08	22	1.86 ± 0.27
	elatel	click beetle larvae	"	3	0.11 ± 0.02	5	1.10 ± 0.10
	fungiv	fungivorous beetles	Lathridiidae, Nitidulidae	22	0.32 ± 0.09	27	21.13 ± 3.67
	lampya	lightning bug adults	Lampyridae	1	0.06	1	2.00
	lampyl	lightning bug larvae	"	4	0.12 ± 0.04	2	1.00
	scarab	scarab beetle adults	Scarabaeidae	10	0.16 ± 0.05	12	1.35 ± 0.14
	stapha	rove beetle adults	Staphylinidae	18	0.17 ± 0.04	24	2.50 ± 0.38
	staphl	rove beetle larvae	"		.	11	1.72 ± 0.29
Diptera	chirol	midge larvae	Chironomidae	1	0.08	11	1.51 ± 0.21
	diptel	dipteran larvae	–	12	0.19 ± 0.05	23	1.90 ± 0.31

[a] Taxa abbreviations (Taxa Code) depicted in redundancy analysis biplots.

[b] Means standardized as number of individuals per trap divided by number of trap days.

[c] Litter extraction conducted using vacuum sampling and berlese method; unit of measure: number of individuals per 0.31 $m^{2.}$

[d] Number of samples in which each taxa was recorded out of 27 total pooled samples.

Table 1.5. Results of Monte Carlo permutation tests examining the relationship between taxa and explanatory variables (via redundancy analysis), with corresponding Figure numbers.

Explanatory Variables (EV)	Sampling Method	Taxa	Trace[a]	*F*	*P*	% Variance[b]		Taxa - EV Corr.[c]		Corresponding Figure No.
						1st Axis	2nd Axis	1st Axis	2nd Axis	
Day vs. Night * Crop Event	Litter Extraction	carabid adults	0.082	1.19	0.102	41.8	33.3	0.626	0.706	—
		bycatch	0.060	1.15	0.192	69.5	16.6	0.801	0.550	—
Sample Method * Crop Event (Capture Efficiency)	Both	carabid adults	0.096	2.03	0.004	61.4	19.8	0.907	0.634	1.4
		all	0.229	5.93	0.004	60.2	33.6	0.875	0.774	1.5
Farm Site Selection	Pitfall Trapping	carabid adults	0.306	1.05	0.018	27.8	20.4	0.814	0.823	1.12
		bycatch	0.326	1.37	0.006	26.7	20.5	0.848	0.870	1.14
	Litter Extraction	carabid adults	0.267	2.27	0.002	35.5	28.0	0.869	0.879	1.13
		bycatch	0.231	2.61	0.002	40.2	22.4	0.805	0.810	1.15
Environmental Attributes	Pitfall Trapping	carabid adults	0.144	1.35	0.022	59.7	31.3	0.810	0.763	1.16
		bycatch	0.129	1.34	0.090	55.0	30.0	0.775	0.733	—
	Litter Extraction	carabid adults	0.085	1.24	0.388	48.4	29.7	0.860	0.775	—
		bycatch	0.102	1.64	0.030	63.6	23.8	0.765	0.594	1.17

[a] The sum of canonical eigenvalues (Trace) defines the total variance explained by the analysis.

[b] Percentage of variance in taxa matrix (observed pattern) accounted for by explanatory variables.

[c] Correlation coefficient (Corr.) of taxa to explanatory variables (EV) for the first and second canonical axes.

Table 1.6. Adult carabid activity density (via pitfall trapping) with biomass measures, body length, and trophic identity assignment.

Species	Trophic ID[a]	Activity Density (AD)[b]	% Total AD	Biomass (mg)[c]	% Total Biomass	Biomass Rank	Median Individual Biomass (mg)	MIB ± SE (mg)[d]	MIB Min / Max (mg)	Median Length (mm)[e]
Harpalus pensylvanicus (DeG.)	omni	131	35.6	4964.3	48.7	1	36.5	37.9 ± 1.1	17.7 - 77.5	15.9
Stenolophus ochropezus (Say)	omni	45	12.2	94.7	0.9	9	2.0	2.1 ± 0.1	0.7 - 4.0	5.9
Amara aenea (DeG.)	omni	33	9.0	207.7	2.0	6	6.1	6.3 ± 0.4	1.1 - 10.7	7.6
Chlaenius tricolor Dej.	carn	23	6.3	463.6	4.5	5	21.6	20.2 ± 2.0	3.4 - 34.5	13.0
Scarites subterraneus F.	carn	18	4.9	921.7	9.0	3	56.9	51.2 ± 5.7	9.5 - 87.1	16.8
Scarites quadriceps Chd.	carn	16	4.3	1929.9	18.9	2	116.0	120.6 ± 6.9	80.1 - 175.8	21.4
Amara familiaris (Duft.)	omni	14	3.8	46.9	0.5	13	3.8	3.3 ± 0.5	0.8 - 6.1	6.7
Tetracha virginica (L.)	carn	10	2.7	826.9	8.1	4	82.0	82.7 ± 3.9	62.4 - 98.0	20.0
Poecilus chalcites (Say)	carn	10	2.7	194.7	1.9	7	19.0	19.5 ± 1.9	11.0 - 29.9	11.6
Elaphropus xanthopus (Dej.)	carn	8	2.2	1.4	<0.1	28	0.2	0.2 ± 0.0	0.1 - 0.3	2.2
Elaphropus anceps (LeC.)	carn	8	2.2	0.9	<0.1	29	0.1	0.1 ± 0.0	0.1 - 0.2	2.4
Acupalpus partiarius (Say)	omni	7	1.9	3.8	<0.1	23	0.6	0.5 ± 0.1	0.2 - 0.8	3.6
Anisodactylus sanctaecrucis (F.)	omni	5	1.4	35.5	0.3	15	7.3	7.1 ± 1.2	3.5 - 10.4	9.3
Bradycellus rupestris (Say)	omni	5	1.4	2.6	<0.1	25	0.2	0.5 ± 0.2	0.1 - 1.1	4.2
Cicindela punctulata Oliv.	carn	4	1.1	66.6	0.7	11	16.4	16.6 ± 0.8	15.1 - 18.6	12.0
Stenolophus conjunctus (Say)	omni	4	1.1	2.4	<0.1	27	0.6	0.6 ± 0.1	0.4 - 0.8	3.9
Dicaelus elongatus Bon.	carn	3	0.8	160.8	1.6	8	55.2	53.6 ± 2.2	49.2 - 56.4	16.8
Amara impuncticollis (Say)	omni	3	0.8	37.3	0.4	14	13.5	12.4 ± 2.2	8.2 - 15.7	8.4
Anisodactylus rusticus (Say)	omni	3	0.8	29.8	0.3	16	6.5	9.9 ± 4.5	4.4 - 18.9	10.5
Harpalus longicollis LeC.	omni	2	0.5	49.7	0.5	12	24.8	24.8 ± 10.6	14.2 - 35.5	13.2
Calathus opaculus LeC.	omni	2	0.5	12.9	0.1	18	6.5	6.5 ± 0.1	6.4 - 6.5	9.1
Agonum punctiforme (Say)	omni	2	0.5	11.2	0.1	19	5.6	5.6 ± 0.1	5.5 - 5.7	7.8

Table 1.6. Adult carabid activity density (via pitfall trapping) with biomass measures, body length, and trophic identity assignment, Continued.

Species	Trophic ID[a]	Activity Density (AD)[b]	% Total AD	Biomass (mg)[c]	% Total Biomass	Biomass Rank	Median Individual Biomass (mg)	MIB ± SE (mg)[d]	MIB Min / Max (mg)	Median Length (mm)[e]
Agonum octopunctatum (F.)	omni	2	0.5	8.3	0.1	20	4.1	4.1 ± 0.3	3.9 - 4.4	7.7
Clivina bipustulata (F.)	omni	2	0.5	8.3	0.1	21	4.1	4.1 ± 0.5	3.6 - 4.6	6.4
Bradycellus tantillus (Dej.)	omni	2	0.5	0.4	<0.1	30	0.2	0.2 ± 0.0	0.2 - 0.3	2.8
Pterostichus atratus (Newm.)	carn	1	0.3	90.9	0.9	10	90.9	.	.	15.5
Patrobus longicornis (Say)	omni	1	0.3	17.5	0.2	17	17.5	.	.	12.0
Selenophorus opalinus (LeC.)	omni	1	0.3	7.8	0.1	22	7.8	.	.	9.2
Notiophilus novemstriatus LeC.	carn	1	0.3	2.8	<0.1	24	2.8	.	.	5.0
Trichotichnus fulgens (Csiki)	omni	1	0.3	2.5	<0.1	26	2.5	.	.	6.5
Polyderis laevis (Say)	carn	1	0.3	0.04	<0.1	31	0.04	.	.	1.3

[a] Trophic identity (Trophic ID) assignment based on literature cited in Appendix E; abbreviations: carn (carnivore); omni (omnivore).

[b] Replication for biomass measures derived from pitfall trapping.

[c] Sum of dry weight for all individuals within species.

[d] Mean individual biomass (MIB) equals the average dry weight of adults.

[e] Species median body length calculated from a subset of field collected individuals.

Table 1.7. Adult carabid density (via litter extraction) with biomass measures, body length, and trophic identity assignment.

Species	Trophic ID[a]	Density[b]	% Total Density	Biomass (mg)[c]	% Total Biomass	Biomass Rank	Median Individual Biomass (mg)	MIB ± SE (mg)[d]	MIB Min / Max (mg)	Median Length (mm)[e]
Stenolophus conjunctus (Say)	omni	600	31.2	442.8	5.4	4	0.7	0.7 ± 0.01	0.2 - 1.4	3.9
Amara familiaris (Duft.)	omni	377	19.6	1987.8	24.1	2	5.2	5.3 ± 0.1	1.1 - 10.2	6.4
Amara aenea (DeG.)	omni	289	15.0	2480.6	30.0	1	8.7	8.6 ± 0.2	2.0 - 15.4	7.6
Acupalpus partiarius (Say)	omni	126	6.5	82.4	1.0	14	0.7	0.7 ± 0.01	0.2 - 1.1	3.6
Stenolophus ochropezus (Say)	omni	73	3.8	181.1	2.2	9	2.5	2.5 ± 0.1	1.3 - 4.6	5.7
Bradycellus rupestris (Say)	omni	67	3.5	58.2	0.7	21	0.9	0.9 ± 0.03	0.3 - 1.5	4.2
Harpalus somnulentus Dej.	omni	66	3.4	615.9	7.5	3	10.4	9.3 ± 0.5	2.2 - 15.8	8.8
Stenolophus rotundatus LeC.	omni	61	3.2	78.3	0.9	15	1.3	1.3 ± 0.05	0.4 - 2.9	4.0
Acupalpus pauperculus Dej.	omni	39	2.0	17.2	0.2	29	0.4	0.4 ± 0.02	0.2 - 0.8	3.1
Trichotichnus fulgens (Csiki)	omni	31	1.6	159.2	1.9	10	5.3	5.1 ± 0.2	2.6 - 9.1	6.7
Elaphropus xanthopus (Dej.)	carn	22	1.1	4.9	0.1	34	0.2	0.2 ± 0.01	0.2 - 0.3	2.2
Anisodactylus rusticus (Say)	omni	21	1.1	295.3	3.6	7	16.6	14.1 ± 1.4	3.3 - 25.0	10.3
Bradycellus tantillus (Dej.)	omni	20	1.0	7.1	0.1	32	0.4	0.4 ± 0.01	0.2 - 0.5	2.9
Agonum punctiforme (Say)	omni	19	1.0	122.7	1.5	11	6.4	6.5 ± 0.5	3.1 - 9.9	7.6
Amara impuncticollis (Say)	omni	17	0.9	189.8	2.3	8	10.0	11.2 ± 1.1	2.3 - 18.6	8.7
Harpalus pensylvanicus (DeG.)	omni	14	0.7	429.7	5.2	5	30.5	30.7 ± 3.4	12.3 - 52.6	15.0
Elaphropus anceps (LeC.)	carn	13	0.7	2.4	<0.1	36	0.2	0.2 ± 0.02	0.1 - 0.3	2.4
Anisodactylus sanctaecrucis (F.)	omni	8	0.4	86.4	1.0	13	11.4	10.8 ± 0.9	5.8 - 13.8	9.2
Calathus opaculus LeC.	omni	8	0.4	62.8	0.8	19	7.7	7.9 ± 0.8	5.2 - 11.6	9.1
Notiophilus novemstriatus LeC.	carn	7	0.4	21.1	0.3	26	3.1	3.0 ± 0.2	2.1 - 4.0	5.0
Agonum octopunctatum (F.)	omni	6	0.3	29.5	0.4	22	3.9	4.9 ± 0.8	3.1 - 8.4	7.6
Polyderis laevis (Say)	carn	6	0.3	0.2	<0.1	42	0.03	0.03 ± 0.003	0.02 - 0.04	1.3

Table 1.7. Adult carabid density (via litter extraction) with biomass measures, body length, and trophic identity assignment., Continued.

Species	Trophic ID[a]	Density[b]	% Total Density	Biomass (mg)[c]	% Total Biomass	Biomass Rank	Median Individual Biomass (mg)	MIB ± SE (mg)[d]	MIB Min / Max (mg)	Median Length (mm)[e]
Poecilus chalcites (Say)	carn	4	0.2	99.4	1.2	12	25.4	24.8 ± 2.9	17.1 - 31.5	11.2
Trechus quadristriatus (Schr.)	omni	4	0.2	2.6	<0.1	35	0.7	0.6 ± 0.02	0.6 - 0.7	3.8
Amara anthobia Vil. & Vil.	omni	3	0.2	10.9	0.1	31	4.3	3.6 ± 1.1	1.4 - 5.2	6.0
Bembidion affine Say	carn	3	0.2	1.2	<0.1	39	0.4	0.4 ± 0.03	0.4 - 0.4	3.1
Poecilus lucublandus (Say)	carn	2	0.1	62.0	0.8	20	31.0	31.0 ± 6.0	24.9 - 37.0	13.3
Amphasia sericea (Harris)	omni	2	0.1	27.0	0.3	23	13.5	13.5 ± 2.1	11.4 - 15.6	9.7
Anisodactylus caenus (Say)	omni	2	0.1	20.7	0.3	27	10.4	10.4 ± 0.2	10.2 - 10.5	9.1
Amara angustata (Say)	omni	2	0.1	14.2	0.2	30	7.1	7.1 ± 3.2	3.9 - 10.3	7.0
Calosoma scrutator (F.)	carn	1	<0.1	377.5	4.6	6	377.5	.	.	35.8
Scarites quadriceps Chd.	carn	1	<0.1	78.1	0.9	16	78.1	.	.	21.4
Scarites subterraneus F.	carn	1	<0.1	68.8	0.8	17	68.8	.	.	16.8
Pterostichus atratus (Newm.)	carn	1	<0.1	64.0	0.8	18	64.0	.	.	15.5
Patrobus longicornis (Say)	omni	1	<0.1	24.7	0.3	24	24.7	.	.	11.9
Chlaenius tricolor Dej.	carn	1	<0.1	23.5	0.3	25	23.5	.	.	13.0
Harpalus affinis (Schr.)	omni	1	<0.1	19.6	0.2	28	19.6	.	.	9.4
Amara cupreolata Putz.	omni	1	<0.1	5.9	0.1	33	5.9	.	.	7.0
Selenophorus pedicularius Dej.	omni	1	<0.1	1.7	<0.1	37	1.7	.	.	6.1
Badister notatus Hald.	carn	1	<0.1	1.5	<0.1	38	1.5	.	.	4.4
Bembidion rapidum (LeC.)	carn	1	<0.1	1.0	<0.1	40	1.0	.	.	4.2
Dyschiriodes globulosus (Say)	carn	1	<0.1	0.3	<0.1	41	0.3	.	.	2.5
Paratachys sagax (Csy.)	carn	1	<0.1	0.1	<0.1	43	0.1	.	.	2.3

[a] Trophic identity (Trophic ID) assignment based on literature cited in Appendix E; abbreviations: carn (carnivore); omni (omnivore).

[b] Replication for biomass measures derived from litter extraction (vacuum sampling and berlese method) for the area 0.31 m^2 (480 $in.^2$).

[c] Sum of dry weight for all individuals within species.

[d] Mean individual biomass (MIB) equals the average dry weight of adults.

[e] Species median body length calculated from a subset of field collected individuals.

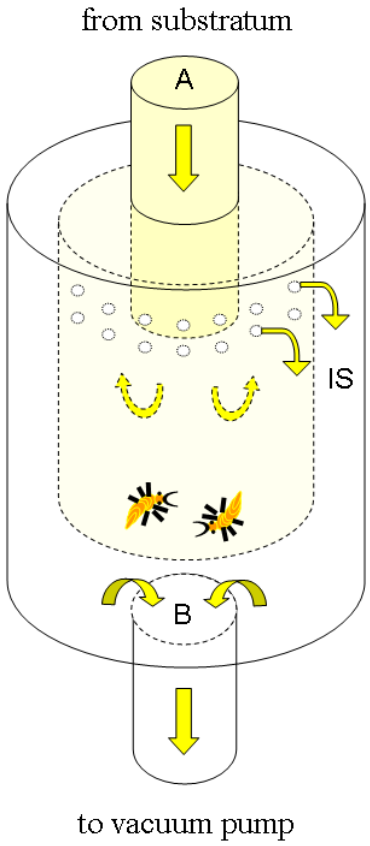

Figure 1.1. Generalized schematic of litter extraction collection chamber. Incoming sample is drawn into the internal collection chamber through input tube (A). Airflow (arrows) from input tube is forced down the center of the internal chamber and subsequently up the inside wall to vents (open circles). Airflow continues out of the collection chamber into the inter-space (IS) between the inner chamber and outer casing, then continues to the inside bottom of the casing, and exits through the output tube (B) to the vacuum pump. Through this mechanism, collected material may be deposited directly into a transfer bucket positioned within the basin of the collection chamber, and/or deposited into a mesh bag secured to the proximal end of input tube (A).

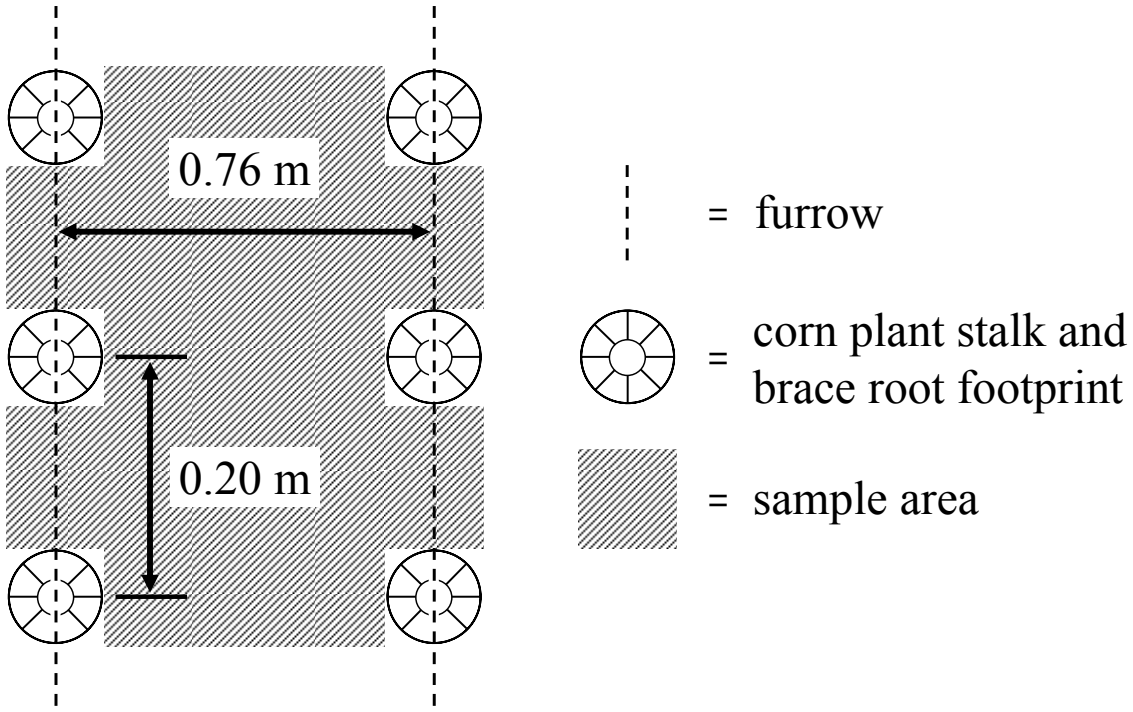

Figure 1.2. Sampling area (≈0.31 m^2 [480 sq in.]) for a single litter extraction sample. Field corn was planted with 0.76 m (30 in.) row spacing and 0.20 m (8 in.) seed spacing within row. Note: not drawn to scale.

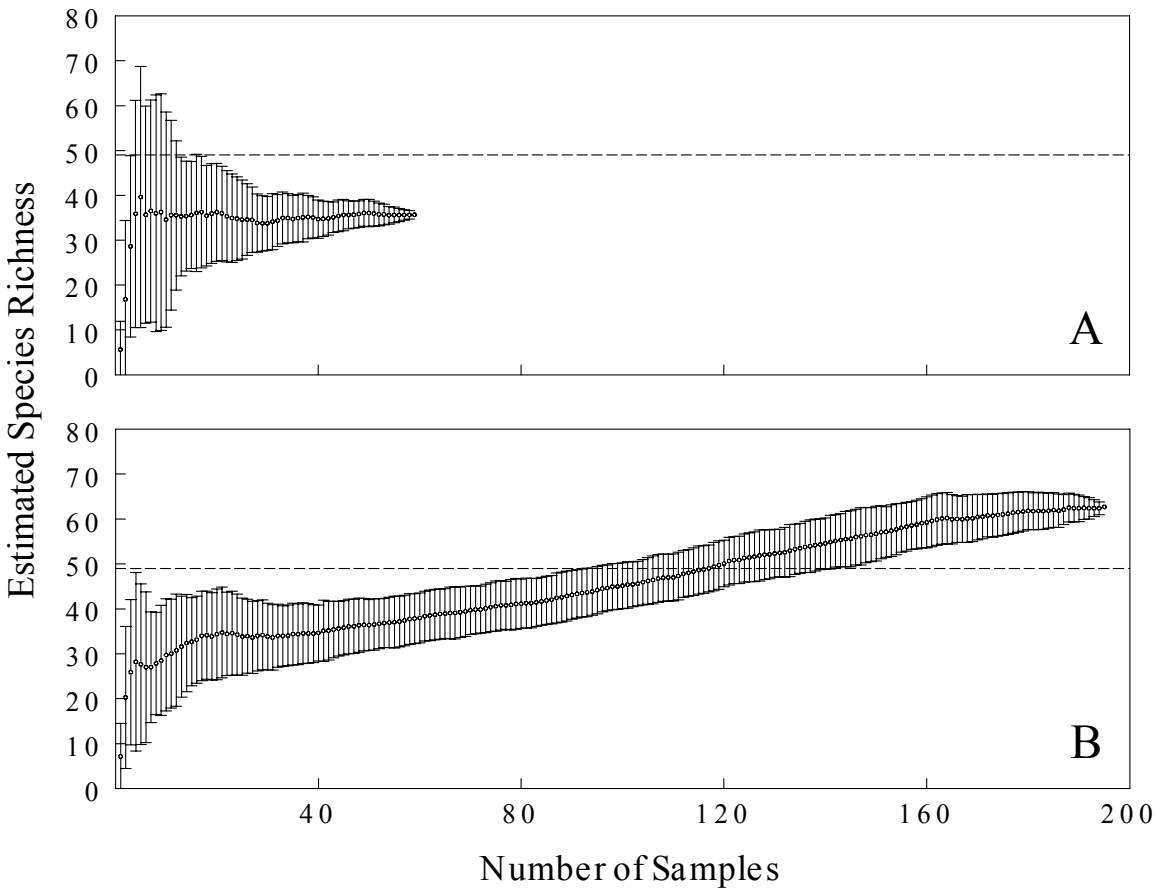

Figure 1.3. Estimation of carabid species richness using the Incidence-based Coverage Estimator (± SD) for: A) pitfall trapping; and B) litter extraction (vacuum sampling and berlese method). Estimated richness values at each level of sampling effort are based on 100 randomizations of the actual data. The hatched line represents the total species richness collected using the combination of pitfall trapping and litter extraction.

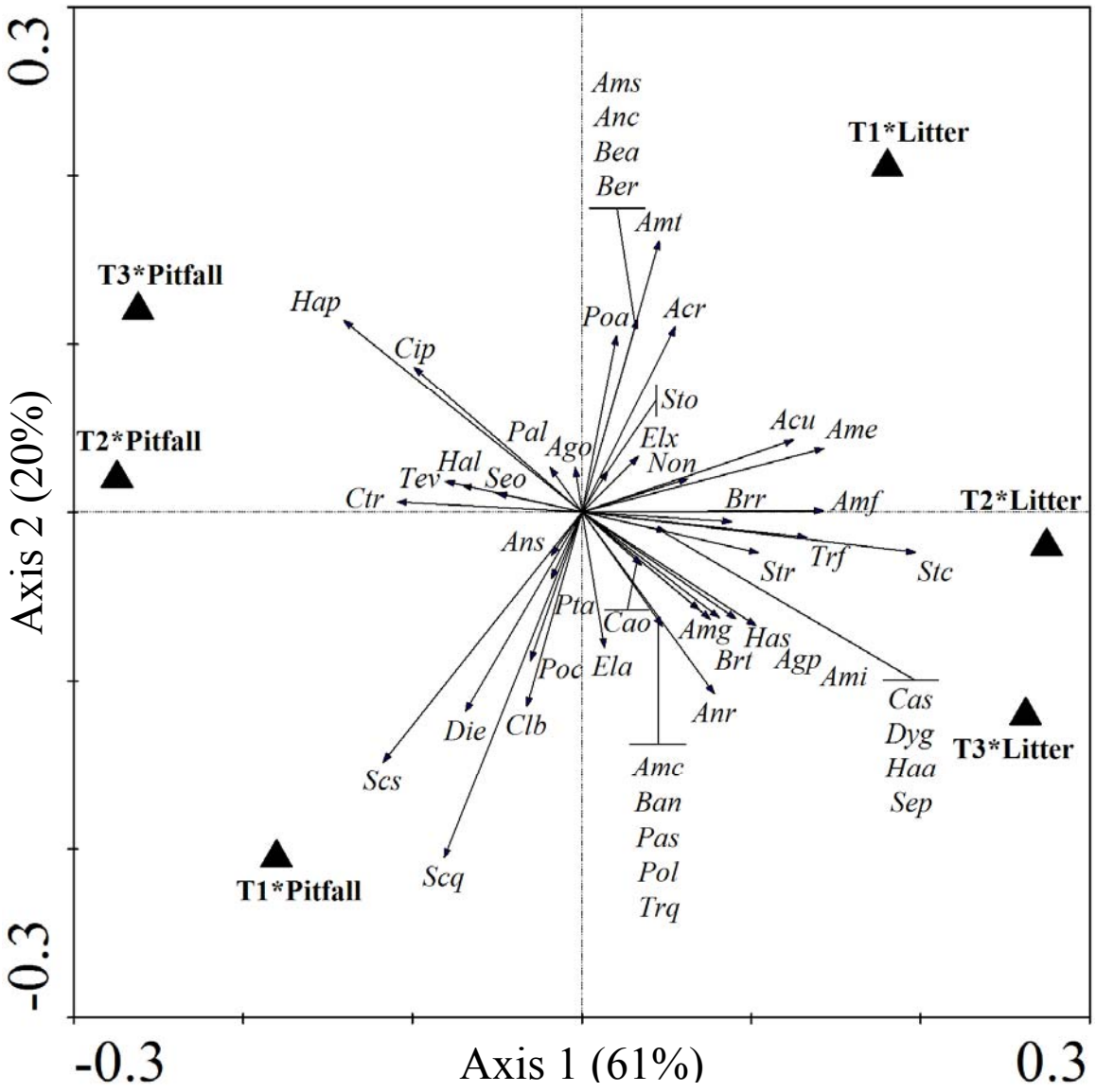

Figure 1.4. Biplot of species capture efficiency of carabid adults for two sampling methods (pitfall trapping [Pitfall]; litter extraction [Litter]) during three cropping events (T1: canopy close; T2: anthesis; T3: post-harvest). A significant interaction of method and cropping event was detected using Monte Carlo permutations ($P = 0.004$). Length of each vector reflects the correlation of species to sampling method for each cropping event. The percentage of trace variance explained by each canonical axis is given. Abbreviations of species names are plotted; complete names are listed in Table 1.3.

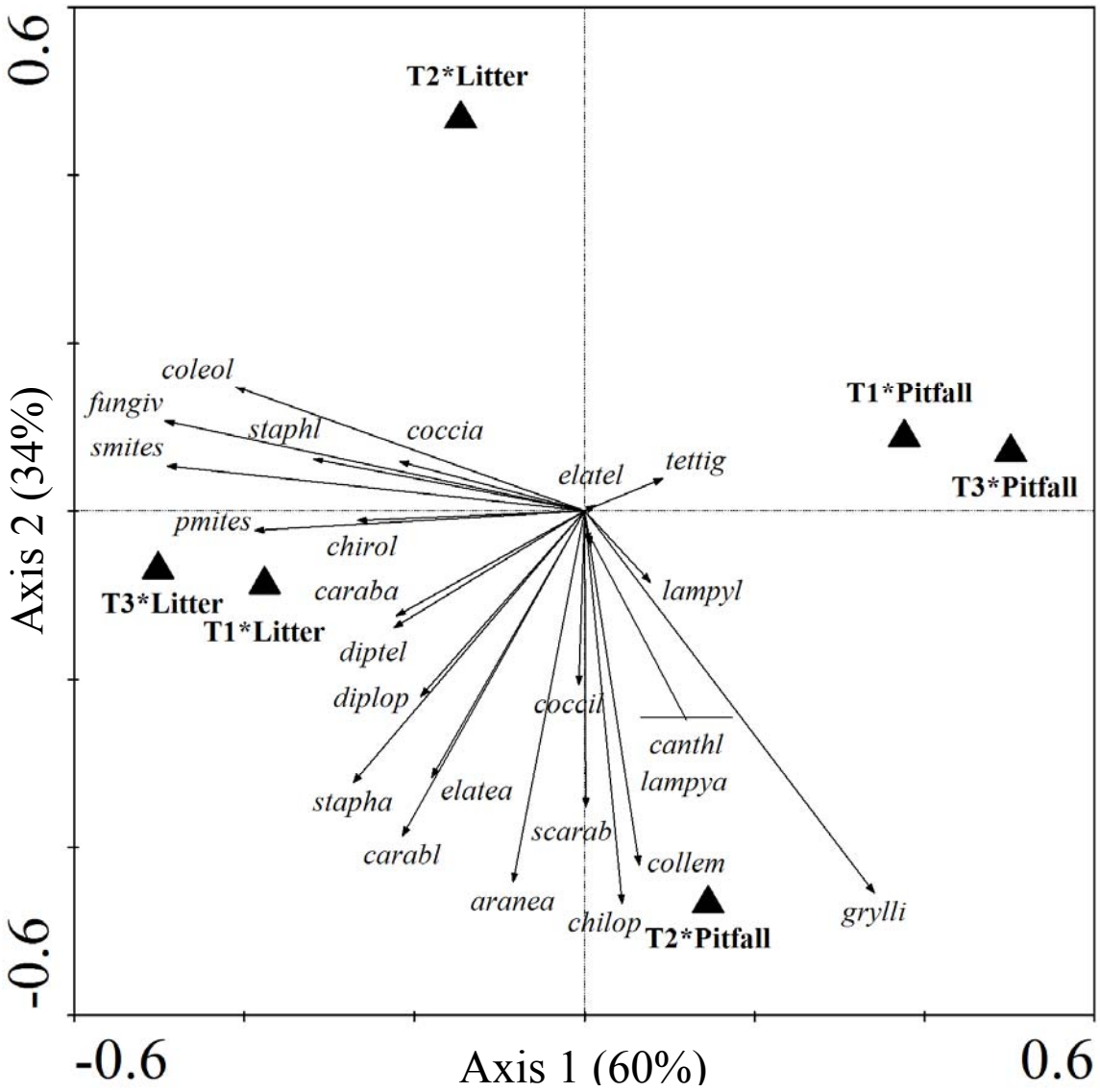

Figure 1.5. Biplot of capture efficiency for all taxa (whole community) for two sampling methods (pitfall trapping [Pitfall]; litter extraction [Litter]) during three cropping events (T1: canopy close; T2: anthesis; T3: post-harvest). A significant interaction of method and cropping event was detected using Monte Carlo permutations ($P = 0.004$). Length of each vector reflects the correlation of species to sampling method for each cropping event. The percentage of trace variance explained by each canonical axis is given. Abbreviations of taxa names are plotted; complete names are listed in Table 1.4.

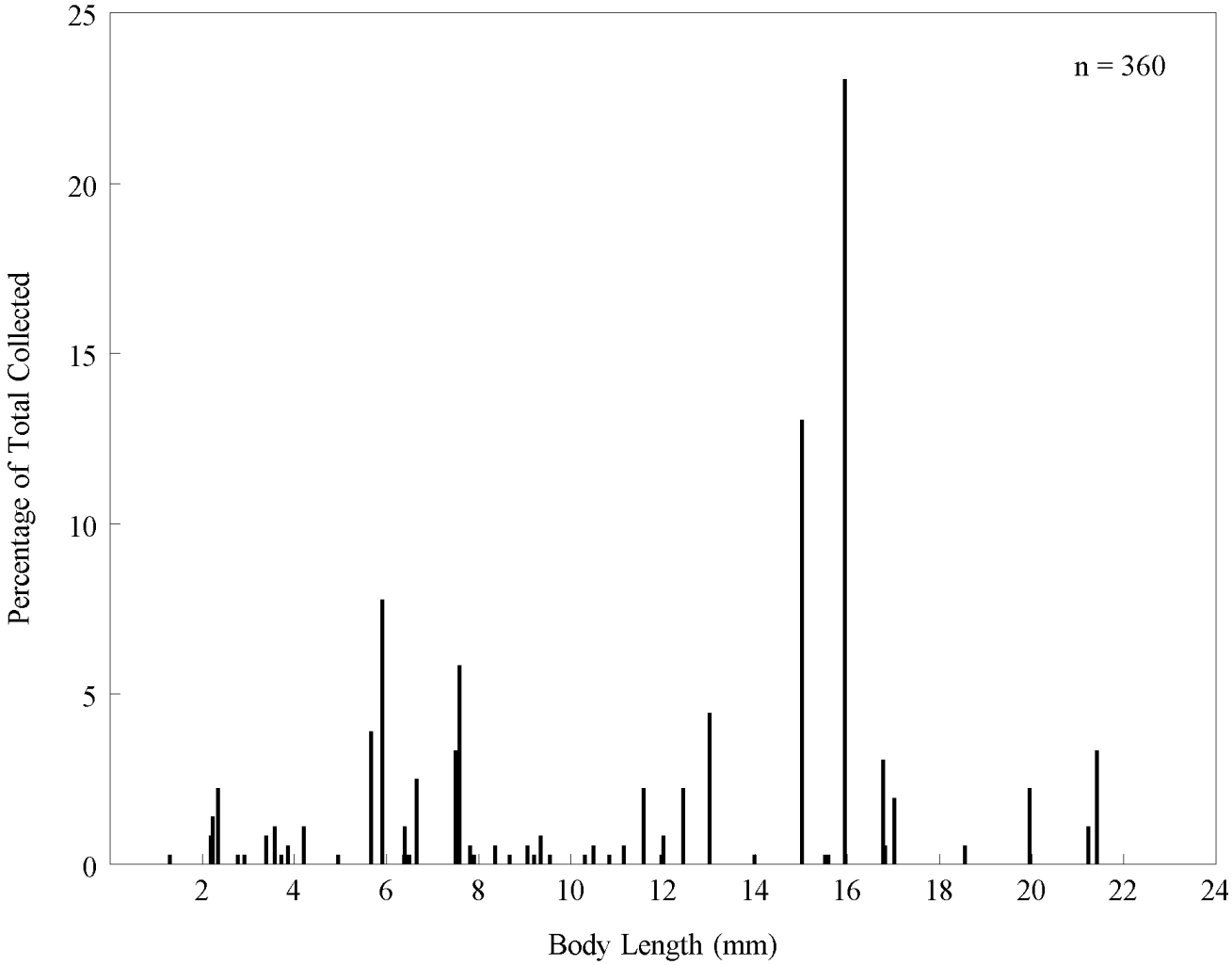

Figure 1.6. Body length distribution for 31 carabid species collected via pitfall trapping. Body length was assigned to each individual from the median value for species by gender from a subset of individuals collected using both pitfall trapping and litter extraction.

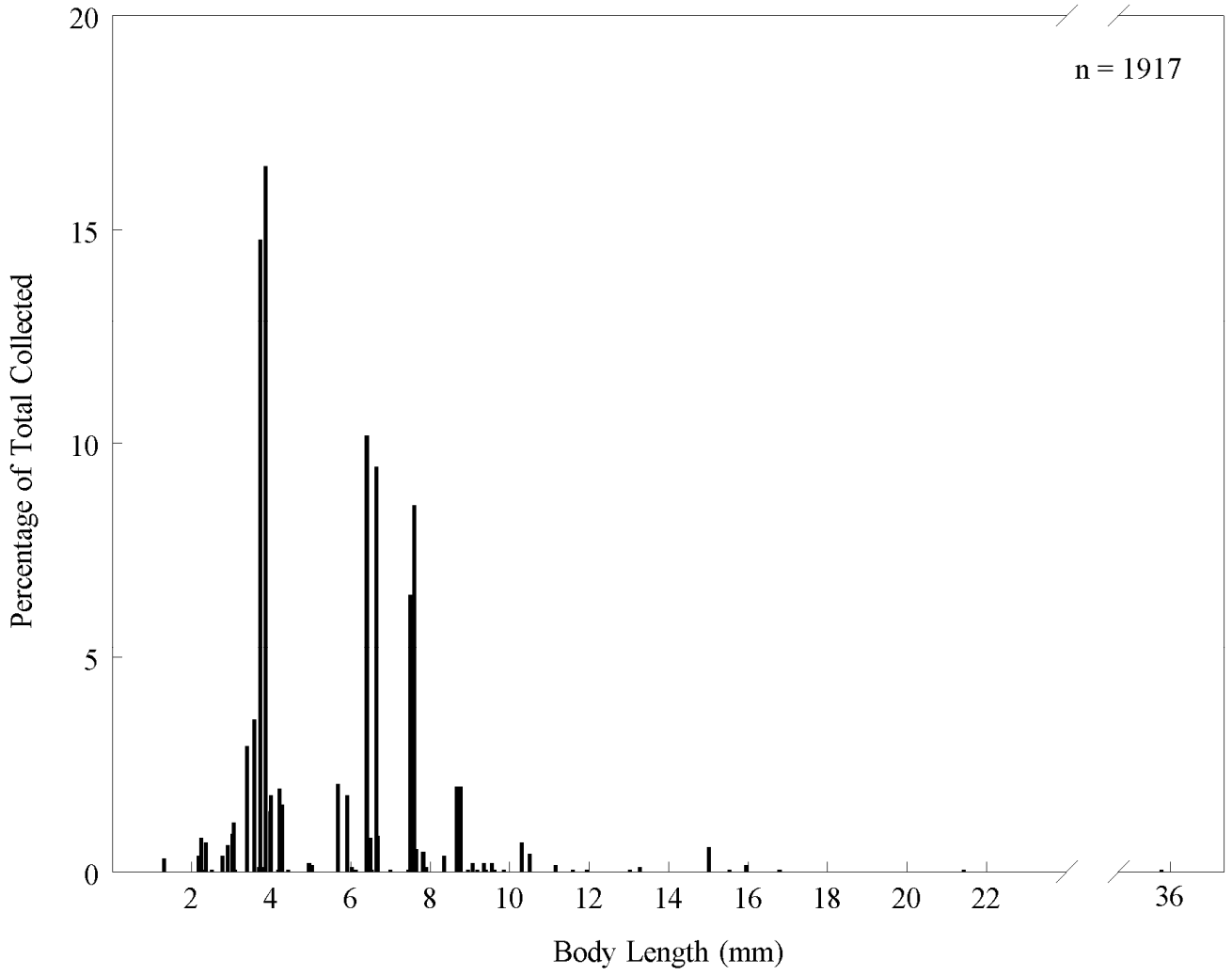

Figure 1.7. Body length distribution for 43 carabid species collected via litter extraction. Body length was assigned to each individual from the median value for species by gender from a subset of individuals collected using both pitfall trapping and litter extraction.

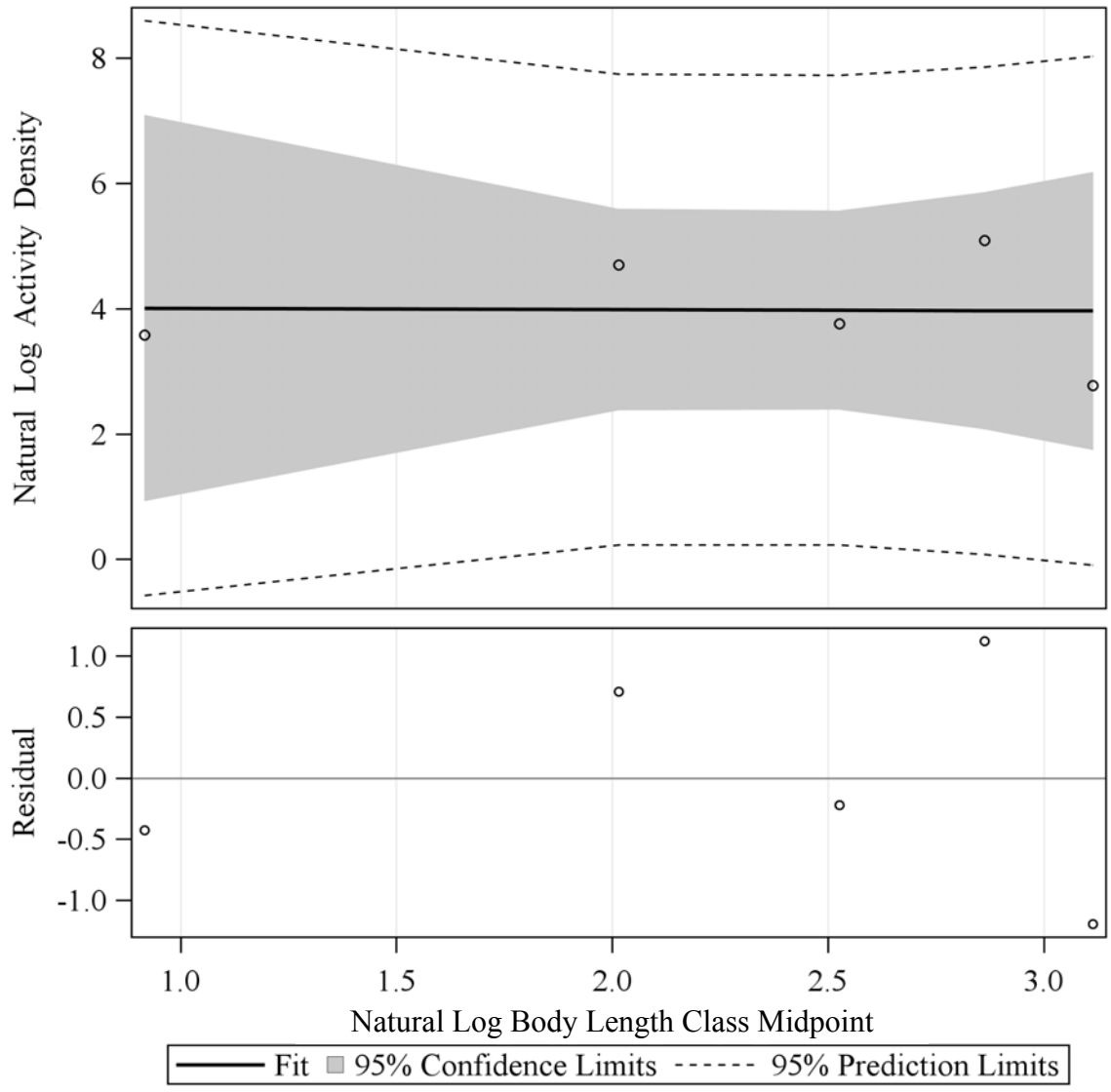

Figure 1.8. General relationship between body length (5 mm classes) and population activity density for carabid adults collected via pitfall trapping. A total of 368 individuals were grouped into 5 mm body length classes, and the class midpoint value was used in analysis. All data was natural log transformed to reduce heteroscedasticity and linearize the model for analysis. The relationship is described by the model ln(activity density) = ln(4.029) -0.021*ln(body length class); R^2 = 0.0004, n = 5, root mean square error = 1.068, P = 0.975.

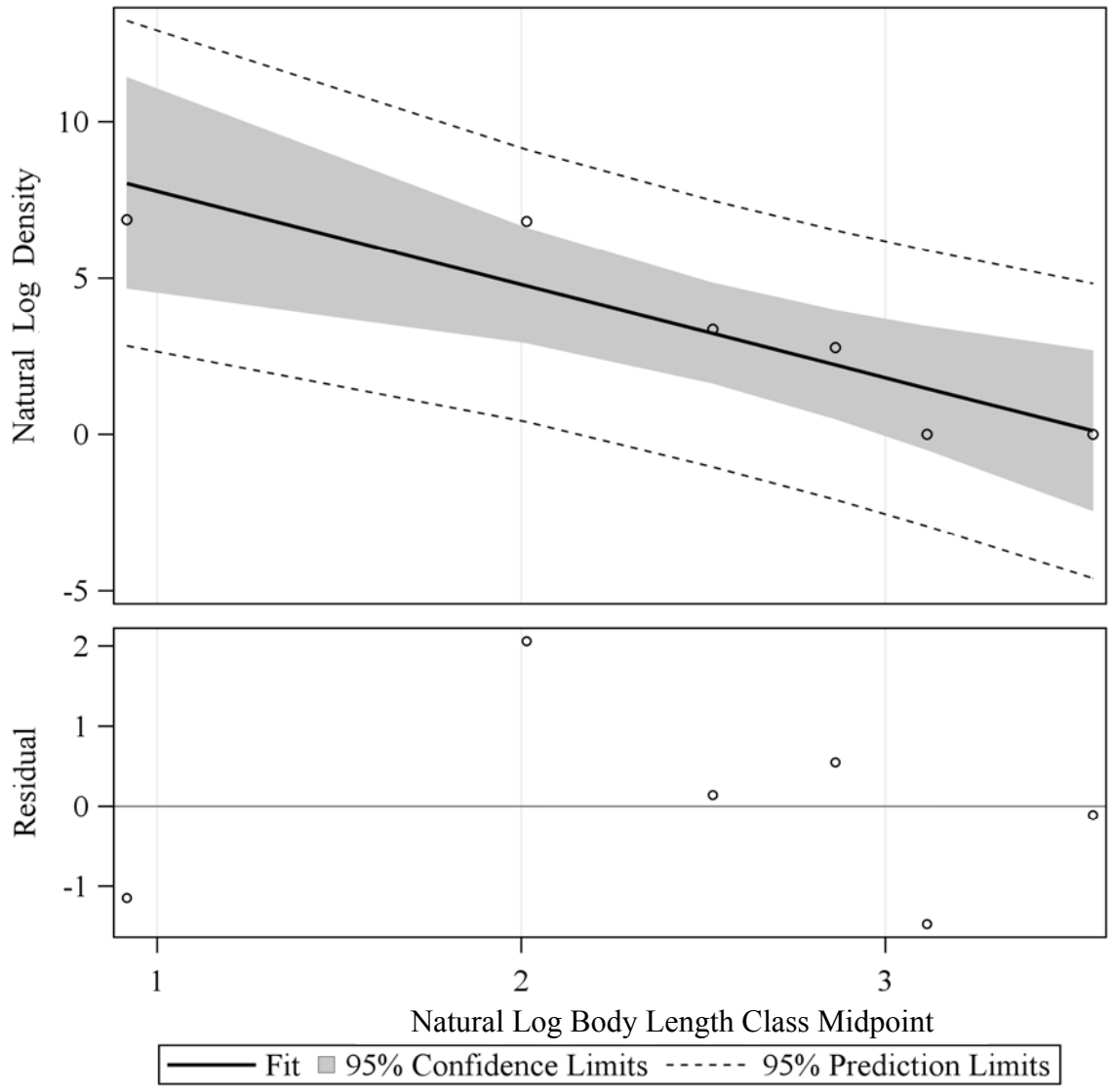

Figure 1.9. General relationship between body length (5 mm classes) and population density for carabid adults collected via litter extraction (vacuum sampling and berlese method). A total of 1925 individuals were grouped into body length classes, and the class midpoint value was used in analysis. All data was natural log transformed to reduce heteroscedasticity and linearize the model for analysis. The relationship is described by the model ln(density) = ln(10.765) -2.983*ln(body length class); $R^2 = 0.829$, n = 5, root mean square error = 1.420, $P = 0.012$. The total sampling area for all litter extractions was 7.75×10^{-2} km^2.

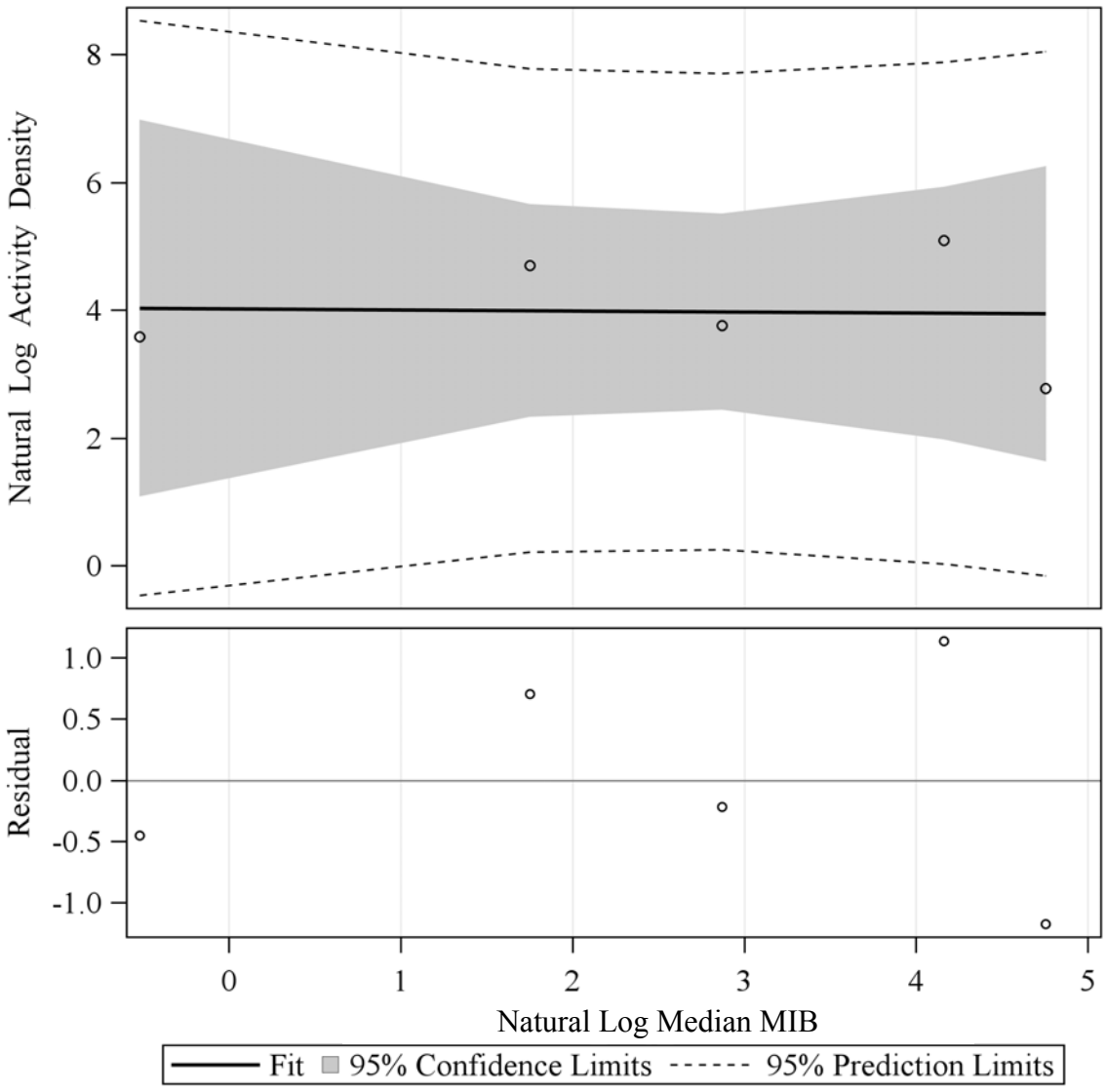

Figure 1.10. General relationship between mean individual biomass (MIB) and population activity density for carabid adults collected via pitfall trapping. A total of 368 individuals were grouped into 5 mm body length classes, and the median MIB within each class was used in analysis. All data was natural log transformed to reduce heteroscedasticity and linearize the model for analysis. The median value of The relationship is described by the model ln(activity density) = ln(4.025) -0.017*ln(MIB); R^2 = 0.001, n = 5, root mean square error = 1.067, P = 0.952.

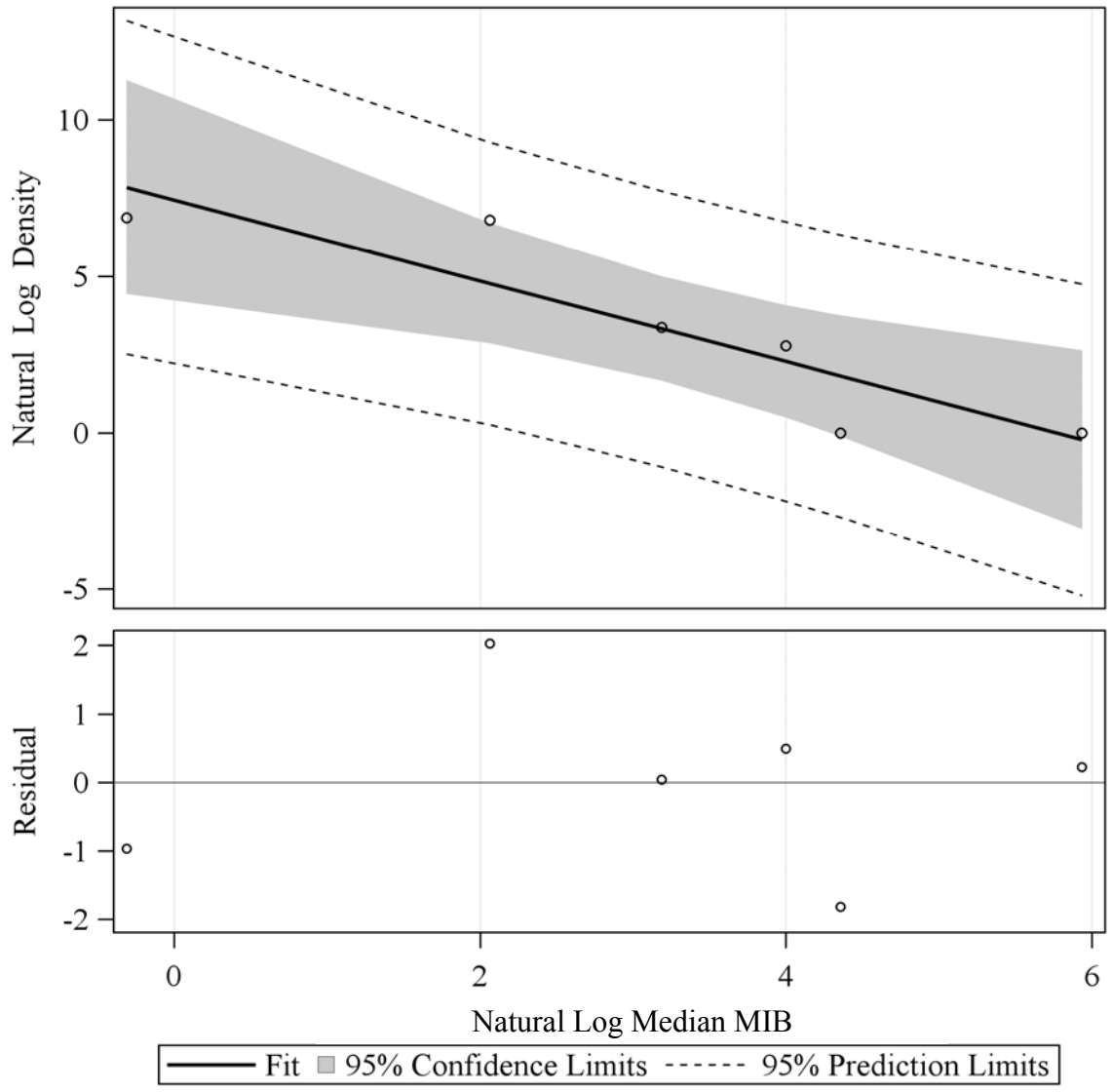

Figure 1.11. General relationship between mean individual biomass (MIB) and population density for carabid adults collected via litter extraction (vacuum sampling and berlese method). A total of 1925 individuals were grouped into 5 mm body length classes, and the median MIB within each class was used in analysis. All data was natural log transformed to reduce heteroscedasticity and linearize the model for analysis. The relationship is described by the model ln(density) = ln(7.448) -1.293*ln(MIB); R^2 = 0.817, n = 5, root mean square error = 1.470, P = 0.013. The total sampling area for all litter extractions was 7.75×10^{-2} km^2.

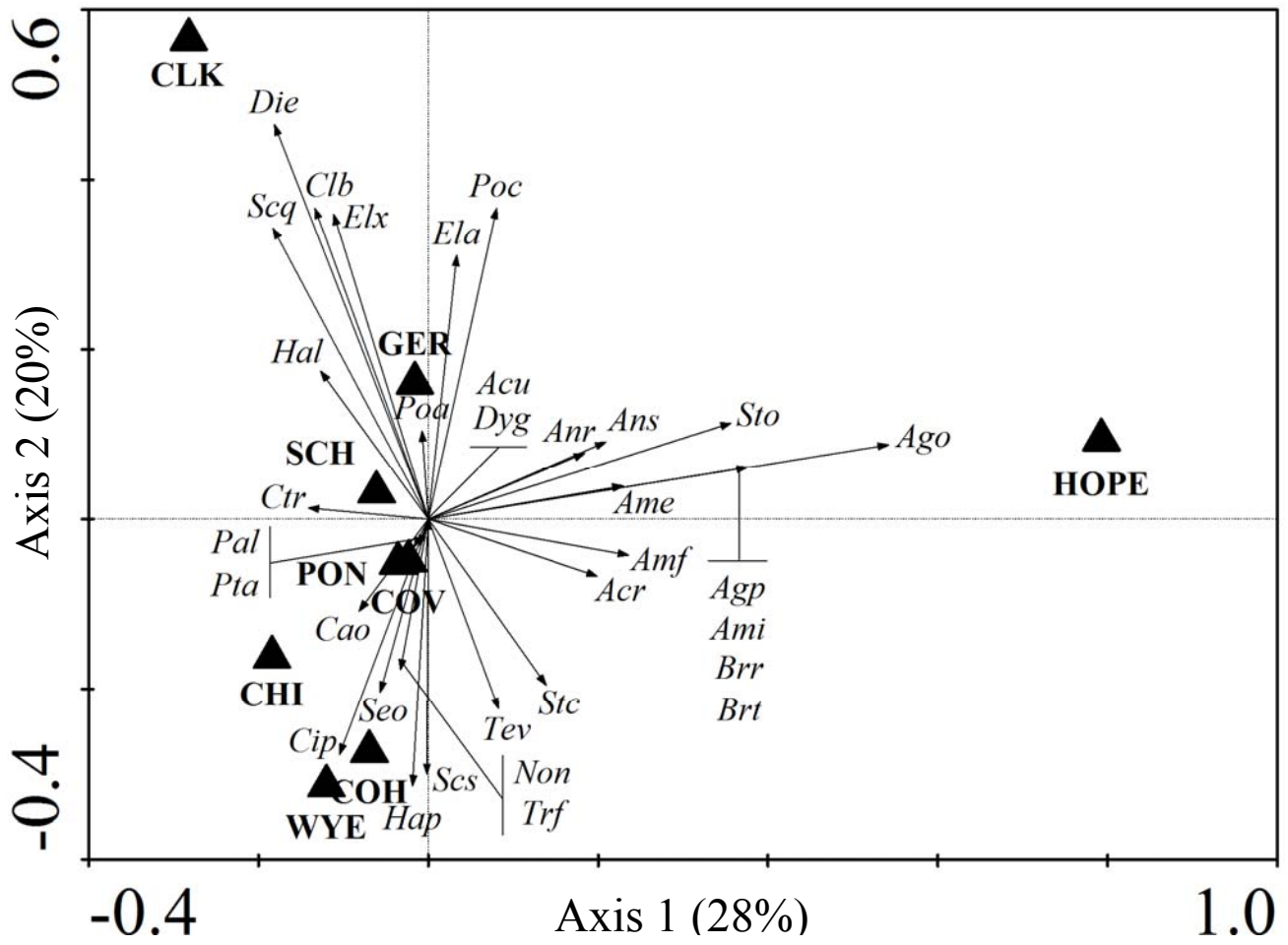

Figure 1.12. Biplot of study site-selectivity for carabid adults collected via pitfall trapping in Maryland field corn. Significant differences between study sites were detected using Monte Carlo permutations ($P = 0.018$). Length of each vector reflects the correlation of species to study site. The percentage of trace variance explained by each canonical axis is given. Abbreviations of study sites and species names are plotted; complete names are listed in Tables 1.2 and 1.3, respectively.

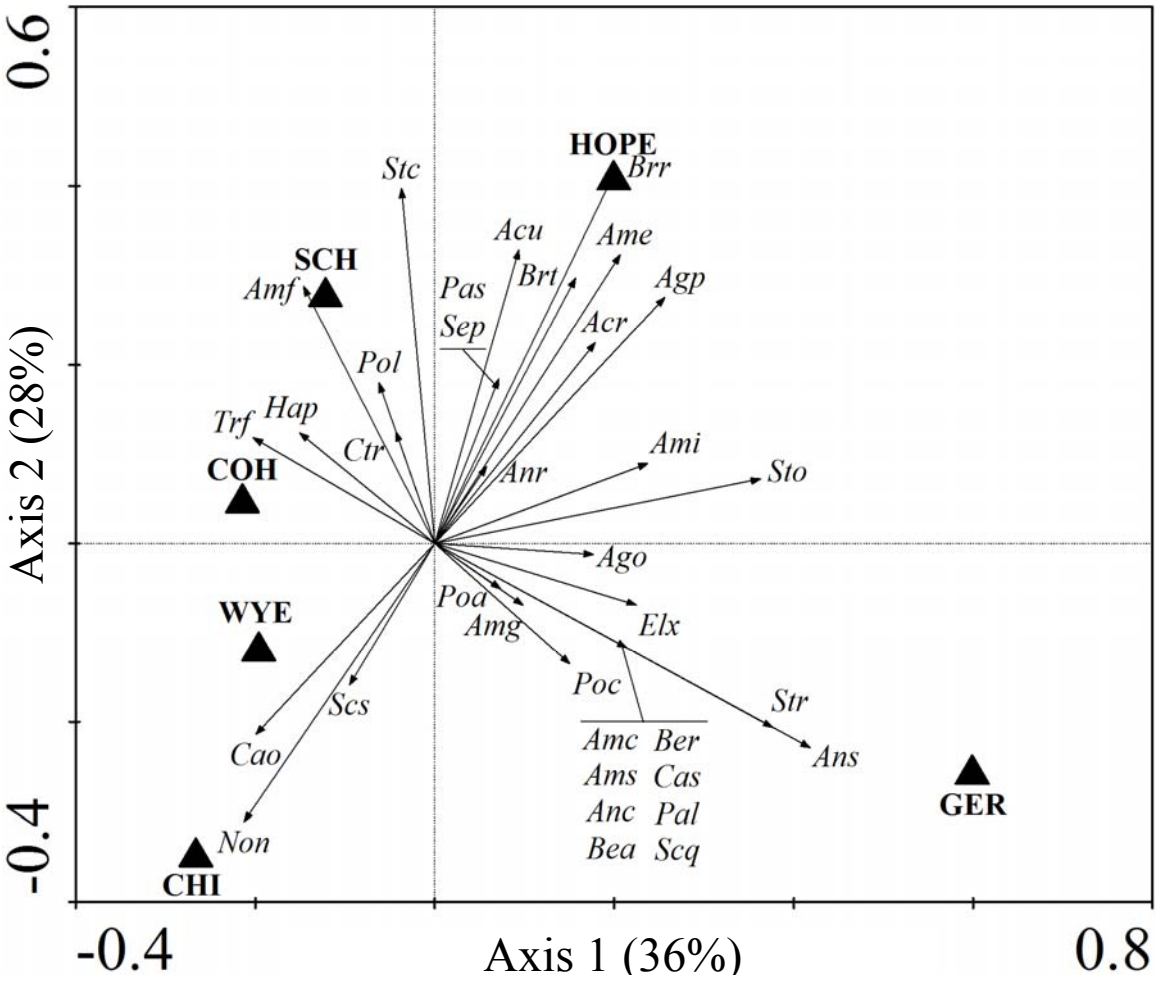

Figure 1.13. Biplot of study site-selectivity for carabid adults collected via litter extraction in field corn systems on the eastern shore of Maryland. Significant differences between study sites were detected using Monte Carlo permutations ($P = 0.002$). Length of each vector reflects the correlation of species to study site. The percentage of trace variance explained by each canonical axis is given. Abbreviations of study sites and species names are plotted; complete names are listed in Tables 1.2 and 1.3, respectively.

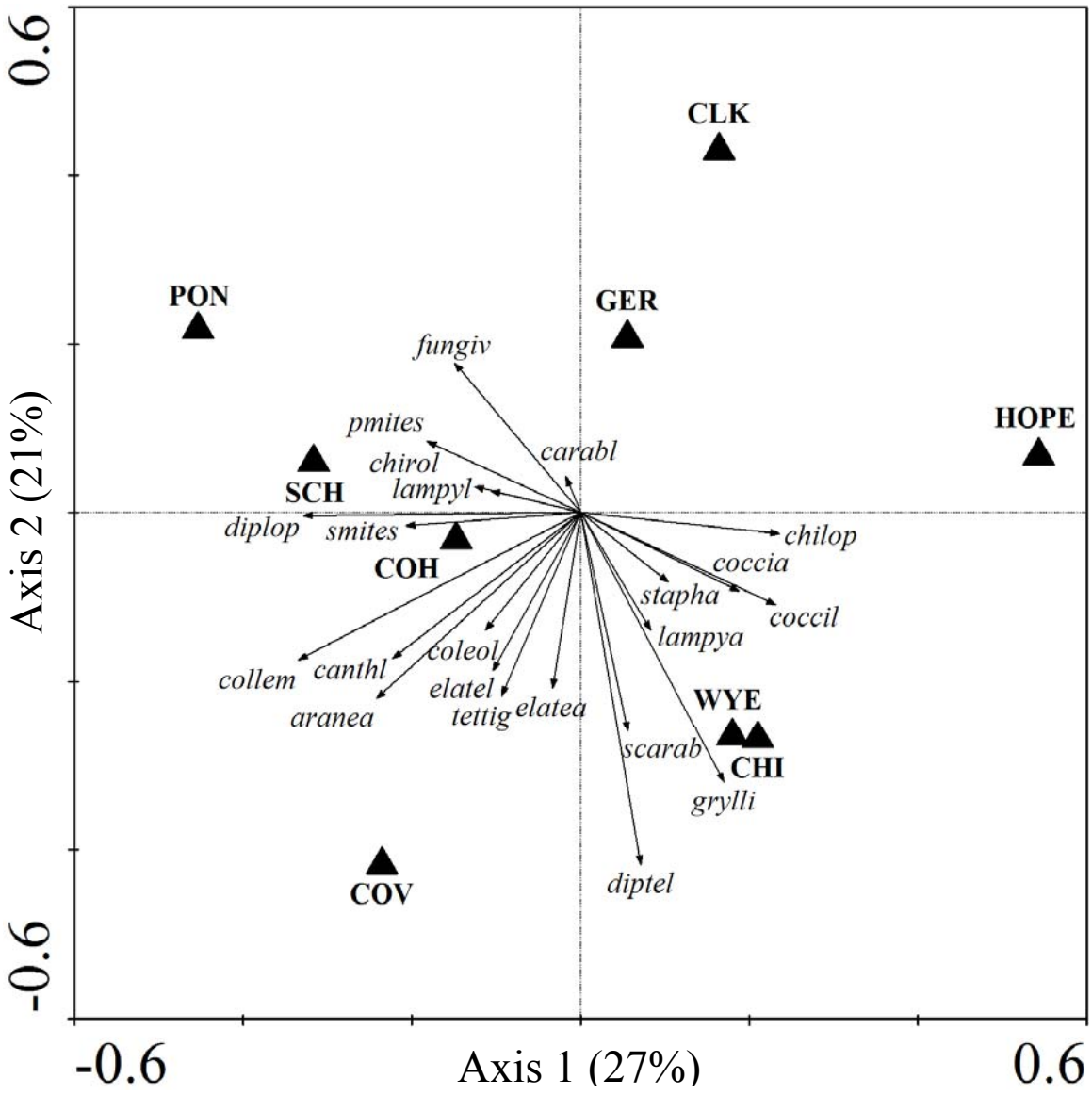

Figure 1.14. Biplot of study site-selectivity for bycatch collected via pitfall trapping in Maryland field corn. Significant differences between study sites were detected using Monte Carlo permutations ($P = 0.006$). Length of each vector reflects the correlation of taxa to study site. The percentage of trace variance explained by each canonical axis is given. Abbreviations of study sites and taxa names are plotted; complete names are listed in Tables 1.2 and 1.4, respectively.

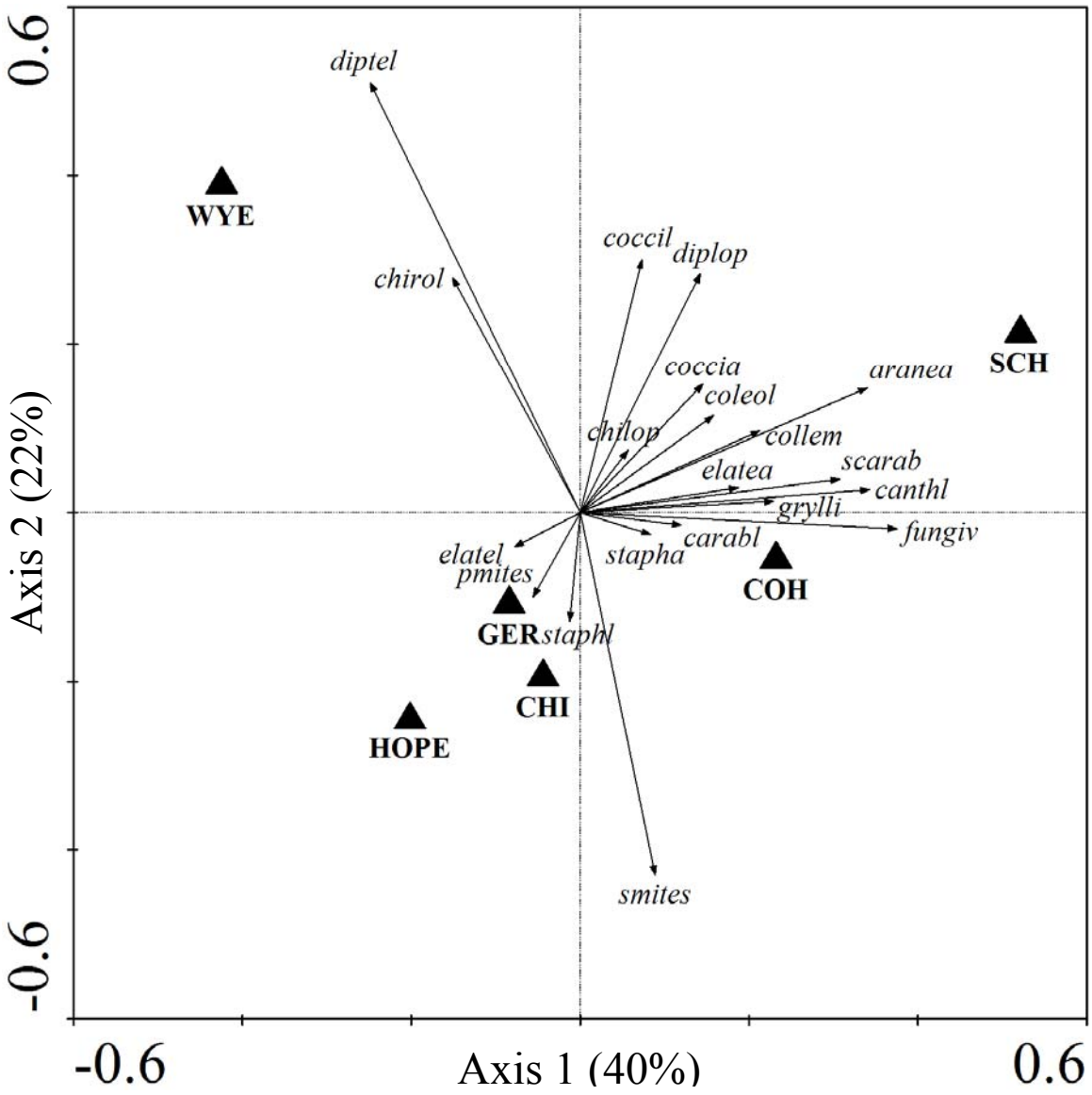

Figure 1.15. Biplot of study site-selectivity for bycatch collected via litter extraction in field corn systems on the eastern shore of Maryland. Significant differences between study sites were detected using Monte Carlo permutations ($P = 0.002$). Length of each vector reflects the correlation of taxa to study site. The percentage of trace variance explained by each canonical axis is given. Abbreviations of study sites and species names are plotted; complete names are listed in Tables 1.2 and 1.4, respectively.

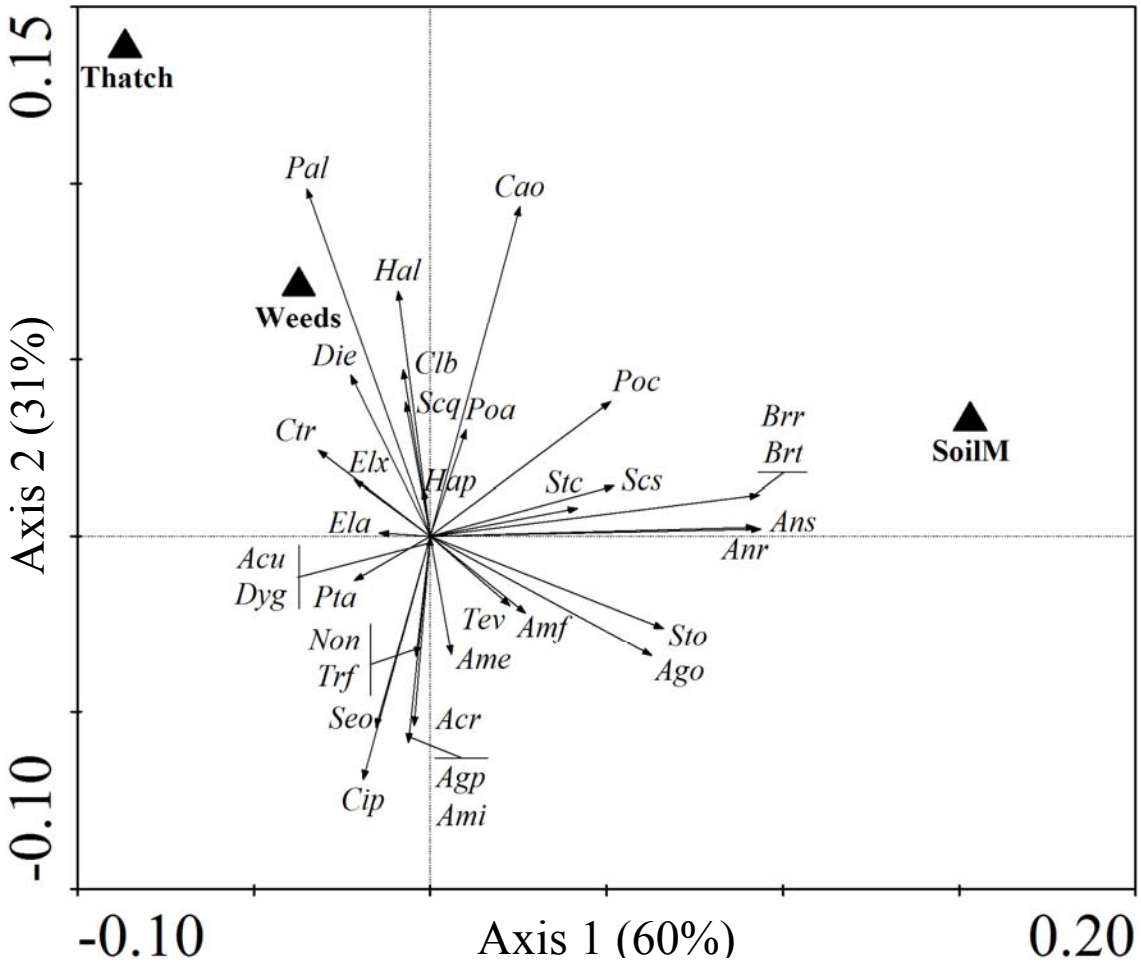

Figure 1.16. Biplot of carabid species correlations to environmental attributes measured in Maryland field corn systems via pitfall trapping. Significant differences between species compositions associated with environmental attributes were detected using Monte Carlo permutations ($P = 0.022$). Length of each vector reflects the correlation of species to environmental attributes (SoilM: soil moisture; Thatch: thatch depth; Weeds: percentage of weed cover). The percentage of trace variance explained by each canonical axis is given. Abbreviations of species names are plotted; complete names are listed in Table 1.3.

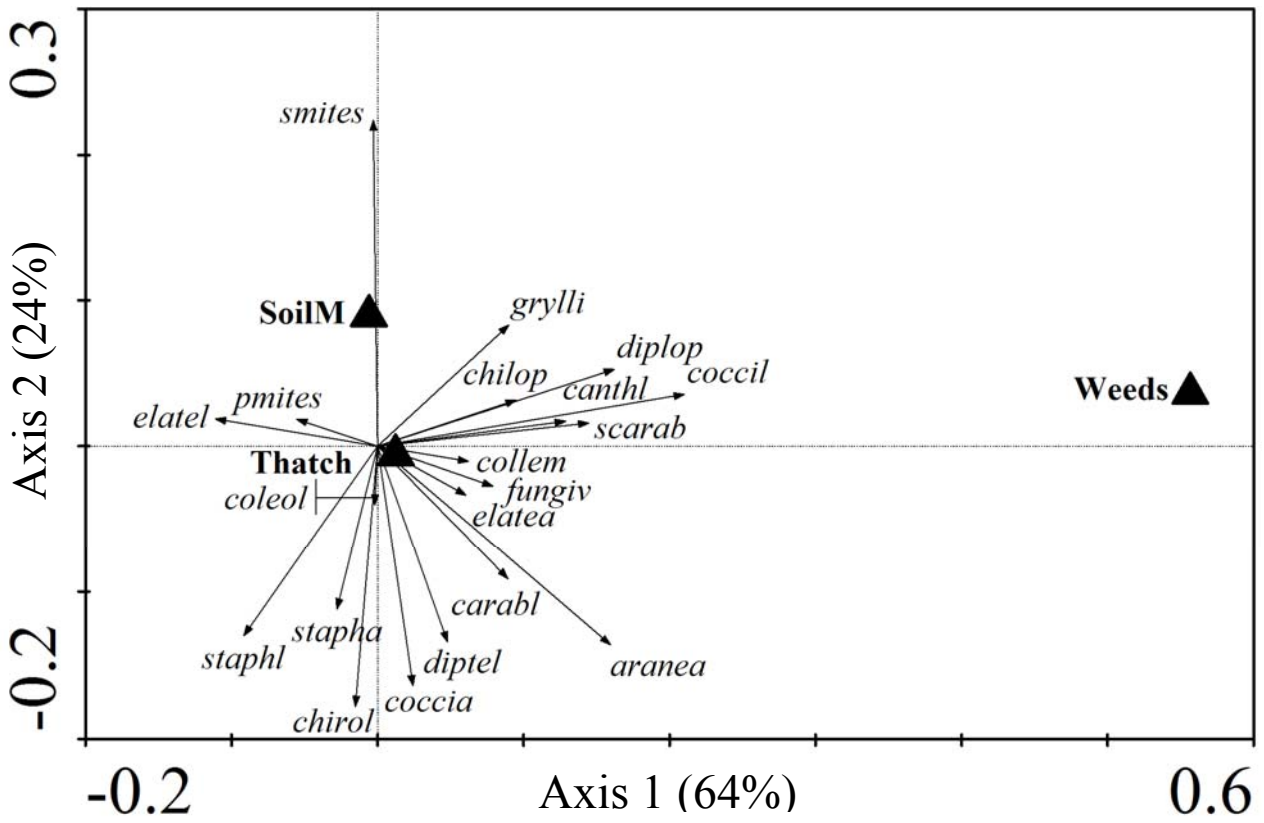

Figure 1.17. Biplot of bycatch correlations to environmental attributes measured in field corn systems on the eastern shore of Maryland via litter extraction. Significant differences between species compositions associated with environmental attributes were detected using Monte Carlo permutations ($P = 0.030$). Length of each vector reflects the correlation of bycatch to environmental attributes (SoilM: soil moisture; Thatch: thatch depth; Weeds: percentage of weed cover). The percentage of trace variance explained by each canonical axis is given. Abbreviations of taxa names are plotted; complete names are listed in Table 1.4.

CHAPTER 2: Estimating diet breadth and exposure routes to rootworm-resistant transgenic corn for a ground beetle, *Elaphropus xanthopus* (Coleoptera: Carabidae)

Abstract

Adults of a ground beetle, *Elaphropus xanthopus* (Dejean) (Coleoptera: Carabidae), were studied in the laboratory to broadly estimate diet breadth and to investigate the potential for exposure to gut-active toxins produced by rootworm-resistant (Cry34/35Ab1) transgenic field corn. No-choice feeding tests examined consumption of plant tissues (anthers, seeds) and animals (collembola, insects, mites, roundworms, slugs) common to agricultural systems in Maryland. Adult *E. xanthopus* readily consumed eggs and early juvenile stages of arthropods, as well as dead microarthropods. The most consumed items included fly eggs and larvae, nematode adults, eggs of collembola and soil mites, and early instar beetle larvae. Of the plant species tested, *E. xanthopus* exhibited little indication of regular herbivory. In choice tests, consumption of transgenic and non-transgenic corn pollen was examined to determine if behavioral avoidance may influence exposure. Additionally, the influence of alternative food availability on exposure estimation was examined through preference tests where varying ratios of pollen cake(s) to fly egg(s) (5:1, 1:1, or 1:5 v:v) were presented. *Elaphropus xanthopus* consumed equal amounts of transgenic and non-transgenic pollen (≈700 grains total). Given a choice of fly eggs and pollen, *E. xanthopus* typically consumed 100% of available eggs and 6% (≈44 grains) of available pollen over the 4 h test period. These findings suggest that *E. xanthopus* may include pollen in its diet, although exposure to transgenic corn pollen may be attenuated by the presence of preferred food items. These results narrow the direct and

indirect pathways through which *E. xanthopus* may be exposed to gut-active transgenic toxins, and suggest this species is primarily carnivorous but may exhibit omnivory via pollen consumption. In working towards knowledge of diet breadth for *E. xanthopus*, these studies utilize a simple statistical adjustment to enable conservative predictions for likelihood of consumption in the field.

Key Words: Likelihood of Consumption Index (LOCI), nontarget organisms, pesticide exposure, transgenic corn

Introduction

With the advent of transgenic plants, agricultural crops may express pesticidal substances continuously during the growing period and subsequently release toxins into the environment during anthesis, senescence, and tissue breakdown following harvest. The majority of transgenic toxins expressed by engineered plants are proteinaceous, gut-active toxins derived from the soil microbe, *Bacillus thuringiensis* (Bt). Root-feeding beetles in the genus, *Diabrotica* (Coleoptera: Chrysomelidae) are serious pests in field corn systems, where they may cost producers $1 billion annually (Metcalf 1986, Gray 2000). Following registration of the first rootworm-resistant Bt corn in 2003, adoption is expected to increase worldwide as *Diabrotica* spp. are widespread in the U.S and have recently established in Europe (Kiss et al. 2005). Global deployment of rootworm-resistant Bt plants may result in nontarget exposure for beneficial or benign coleopteran species. For nontarget predatory beetles, exposure to transgenic proteins may potentially occur through direct feeding on plant tissues (Lundgren and Wiedenmann 2002, Moser et al. 2008) or exudates (Saxena et al. 2002), and through indirect consumption of intoxicated prey (Harwood et al. 2006, Zurbrügg and Nentwig 2009) or bioaccumulator organisms (Zhang et al. 2006). Recently, ground-dwelling beetles in the family Carabidae have received attention in nontarget testing programs examining exposure to Bt proteins in the laboratory and field (Zwahlen and Andow 2005, Harwood et al. 2006, Álvarez-Alfageme et al. 2009, Peterson et al. 2009).

In a variety of natural and managed systems, carabid beetles have been utilized as biological indicators (bioindicators) of habitat quality and environmental disturbance (Maelfait et al. 1990, Luff 1996, McGeoch 1998, Kromp 1999, Rainio and Niemelä 2003). Additionally, carabids are recognized as natural enemies of crop pests (Kromp 1999), via specialist relationships but more commonly through opportunistic consumption of palatable species (Lövei and Sunderland 1996, Symondson et al. 2002). Carabids are known to eat gastropods, fruit, live and dead arthropods (Larochelle 1990), seeds (Lundgren 2009), fungal tissue (Johnson and Cameron 1969, Allen 1979), and may indirectly consume pollen (Mitchell 1963, Dawson 1965). While carabids are often labeled generalist predators, several species may be considered generalists with specialist tendencies, by consuming opportunistically but exhibiting strong food preference (Larochelle 1990). Studies have also highlighted the importance of examining carabid feeding behavior at the species-level (Barney and Pass 1986), rather than relying on generalizations at the family- or genus-level. Nontarget studies for Bt toxins have focused on large-bodied, primarily carnivorous species (e.g. *Lebia grandis*, Riddick and Barbosa 2000; *Poecilus chalcites*, Duan et al. 2006; *P. cupreus*, Heimbach et al. 2000, Álvarez-Alfageme et al. 2009; *Scarites subterraneus*, Harwood et al. 2006). Large-bodied carnivores may not consume significant quantities of Bt proteins if indirect exposure pathways predominate, as prey tissues may effectively dilute Bt exposure (Harwood et al. 2006). Conversely, direct exposure routes, including consumption of anthers, pollen, and exudates, may be expected to result in higher exposure and the potential for adverse effects. For example, during anthesis in corn, pollen availability may exceed prey availability, as corn pollen grain densities may reach 500 grains cm^{-2} (Smith et al. 2004)

to 1000 grains cm^{-2} at ground level (M.D. Lepping, unpubl. data). Although there are no known cases of ground beetles that feed exclusively on pollen, facultative feeding or unintended uptake of corn pollen is expected for smaller species, as: (1) small-bodied *Bembidion quadrimaculatum oppositum* and larger species consume corn pollen in the laboratory (Mullin et al. 2005); (2) gut dissections have revealed non-corn pollen consumption (Mitchell 1963, Dawson 1965); (3) select small-bodied carabid species have been found to test positive for Bt proteins during anthesis (*Elaphropus xanthopus*; Chapter 3), and larger species have tested positive during anthesis, but not before (*Poecilus cupreus*; Álvarez-Alfageme et al. 2009); and finally, (4) small-bodied species that exhibit egg predation could naturally switch to corn pollen during periods of high pollen availability. While some pollen feeding is expected, preference for food resources other than corn pollen would be expected to influence exposure. Additionally, the ability to distinguish between transgenic and non-transgenic pollen may influence exposure, whether pollen is encountered in a field planted with or without a transgenic variety, or planted with a mixture of transgenic and non-transgenic seed (e.g. refuge border, refuge-in-the-bag). Carabid species nominated as candidate bioindicators would ideally be exposed to transgenic proteins through limited pathways and in high doses relative to body size. These criteria may be satisfied through identification of carabid species that exhibit a narrow diet breadth, are capable of feeding on fresh corn tissues or exudates, and do not avoid palatable transgenic tissues in the presence or absence of choice.

Understanding the feeding preferences of individual carabid species enables: (1) assessment of bioindicator utility through establishing pathways of exposure to

environmental toxins; and (2) linking of predator-prey relationships and estimation of biological control potential. The main objective of this study was to broadly describe the diet breadth of a ground beetle, *Elaphropus xanthopus* (Dejean) (Coleoptera: Carabidae), and to examine its potential for use as a bioindicator species in nontarget risk assessment programs. Specific objectives included examination of trophic identity through consumption of pest and non-pest species, potential exposure routes for proteinaceous transgenic toxins, and the influence of alternative foods on consumption of transgenic corn pollen.

Study organism

Laboratory studies utilized a ground beetle, *Elaphropus xanthopus* (Dejean) (Coleoptera: Carabidae: Trechitae: Bembidiini: Tachyina) (formerly *Tachys xanthopus*) (Dejean 1831, Bousquet and Larochelle 1993, Ciegler 2000). Thought to be an endemic species, *E. xanthopus* distribution includes most of eastern North America, but confirmed reports are limited to areas east of Texas (Bousquet and Larochelle 1993). *Elaphropus xanthopus* is small-bodied carabid (1.7-2.4 mm adult body length) (Ciegler 2000) and fully winged. Although macropterous, *E. xanthopus* may be classified as an occasional flier; exhibiting seasonal flight in the field and flight in the laboratory only under starving conditions (M.D. Lepping, pers. obs.). *Elaphropus xanthopus* and congeneric species have been reported from lacustrine and riparian habitats (e.g. *E. parvulus*, LaBonte and Nelson 1998), as *E. xanthopus* favors mud flats (Boerner 1894, Blatchley 1910), and in dry years is often located in areas of moist soil (M.D. Lepping, pers. obs.). Within Maryland, *E. xanthopus* inhabits urbanized turf- (M.D. Lepping, unpubl. data) and agro-ecosystems

(Chapter 1). *Elaphropus xanthopus* adults are active in corn systems from April until September in Maryland (Chapter 1), and may be collected in South Carolina from April until August, and in November (light trapping and/or tanglefoot screen trapping; Ciegler 2000). *Elaphropus xanthopus* is likely univoltine, and is gravid in spring following overwintering in the adult stage (M.D. Lepping, unpubl. data), as do other *Elaphropus* species (Erwin 1981). Area-standardized litter extractions in no-till field corn plots ranked *E. xanthopus* as the most abundant species after the seed-feeding genera (e.g. *Stenolophus*, *Amara*, *Acupalpus*, *Bradycellus*, *Harpalus*, *Trichotichnus*) (Chapter 1). Diet information has not been previously reported for *E. xanthopus*. Examination of *E. xanthopus* mandibles (Figure 2.1) suggests carnivory over omnivory (*senso lato* granivory), as the length to width ratio is high (Forsythe 1983), and the presence of a long terebral ridge is characteristic (Frank 2007). Limited observations have been reported for some congeneric species, including: *E. anceps*, which may consume soybean aphid (*Aphis glycines*) in captivity (Rutledge et al. 2004); *E. incurvus*, a myrmecophilous species which may consume dead or disabled ants in the field, and anthomyiid fly eggs in captivity (Larochelle 1990); and *E. vernicatus*, which feed on plants in the Brassicaceae (Larochelle & Larivière 2003). These life history characteristics suggest *E. xanthopus* may be an appropriate bioindicator species.

Materials and Methods

Beetle collection

Elaphropus xanthopus adults used in studies were collected from non-transgenic field corn plots at the U.S. Department of Agriculture, Beltsville, MD (USDA-ARS-BARC) and a commercial farm on the eastern shore of Maryland (Queen Anne's County). Specimens were collected using aspirators and vacuum sampling. Beetles were maintained at 20°C in colony vials with moist filter paper, and were given access to ground dog food (IAMS Smart Puppy; IAMS Co., Dayton, OH) for 24 h, then starved for 24 h prior to experiment.

No-choice tests

No-choice feeding tests were conducted throughout the year when *E. xanthopus* adults coincided with potential prey. In total, 35 selective treatments of 15 species of plants and animals as prey were tested in a nested incomplete factorial design, arranged in randomized complete blocks. The number of replicates varied from 2 to 40 depending on the test item (Table 2.1). Treatments included test item life-stage (e.g. egg/seed, immature stage/instar, adult), state (dead, alive, viable) and quantity (1, 5, 20), nested within test item species (Table 2.1). Test item size was measured to examine its relationship to beetle consumption. Test items were selected to serve as representative taxa for groups that inhabit agro-ecosystems. Plant and animal test item acquisition and preparation are detailed in Appendix J.

To limit animal escape, studies were conducted using tight-sealing Petri dishes (50 x 9 mm; 351006, BD Falcon™ Tight-fit Lid Dish, Becton Dickenson Biosciences Discovery Labware, Bedford, MA). Five of each test item were added to a Petri dish, with the exception of single slug adults, as a means to increase the likelihood of encounter, and therefore the opportunity for consumption or rejection. Test items were arranged in a pentagon formation within each Petri dish, with each item approximately 1 cm from the dish edge. All treatments were acclimated to 25°C for a minimum of 1 h prior to beetle introduction. At the beginning of a feeding trial, each dish received one adult *E. xanthopus*. To prevent escape in treatments with highly mobile organisms (e.g. adult and juvenile collembola), *E. xanthopus* were transferred into Petri dishes using a large eye dropper filled with dH_2O. Once sealed, all dishes received ≈500 µL of dH_2O, applied to the filter paper rim, and were contained in resealable plastic bags.

Tests were conducted in a single growth chamber at 25°C, beginning in the dark phase of a 16:8 h (light:dark) photoperiod. Test item consumption was recorded at 4 h. Examination of later time points revealed changes in test item state (e.g. egg hatch, cannibalism, pupation, decay), and little additional consumption after 4 h. Accordingly, time points after 4 h were not included in analysis, with one exception. In the live 1st instar collembolan treatment, by 16 h all had died, and *E. xanthopus* consumption of dead individuals was recorded and included in analysis. Under a stereomicroscope, data were recorded as the sum of proportions remaining of all test items present. Proportions were expressed in quartile increments, except for seeds which were recorded in increments of 0.05. To aid in quantification, test items in dishes with beetles were compared against

reference test items in dishes without beetles. Consumption (proportion eaten) was defined as the difference of the number of test items presented and the sum of proportions remaining at 4 h. If a proportion of any test item was missing, the associated beetle was recorded as having fed.

Choice tests

Choice tests were conducted to determine if *E. xanthopus* distinguished between Bt and non-Bt corn pollen, and to examine the influence of alternative food availability (frequency of each food item) on pollen consumption. These tests were conducted during late-July when anthesis and *E. xanthopus* adults coincided. Pollen-only choice tests were arranged as a completely randomized design where Bt and non-Bt pollen cakes alternated in a circle within each Petri dish. Food availability tests were arranged as a randomized complete block design with a 2x3 factorial treatment structure. Factors included two levels of pollen type (Cry34/35Ab1 or non-Bt near isoline; Dow AgroSciences, Indianapolis, IN) and three levels of food availability (frequency), where each level varied in the ratio of pollen cake(s) to *Drosophila melanogaster* (Meigen) egg(s) (5:1, 1:1, or 1:5 v:v). Pollen cakes equal in volume to *D. melanogaster* eggs were formed by pipetting 3 μL aliquots of a 0.04 g mL^{-1} pollen solution onto filter paper discs (≈240 grains per cake). Visual observations were recorded at 4 h for 20 replicates of the pollen-only tests; in food availability tests, 20 replicates were completed for each level of pollen type (Bt or non-Bt). Consumption was measured in quartile increments as per the no-choice tests.

Statistical analysis

No-choice feeding tests

Differences in consumption of test material (proportion eaten) by *E. xanthopus* in no-choice tests were examined using ANOVA (PROC MIXED; SAS Institute Inc. 2008), with unbalanced replication (Table 2.1). All tests conducted throughout the experimental period were pooled into a single analysis, accounting for random effects (time of year, block). Tukey-Kramer tests were used to limit experimentwise error rates ($\alpha = 0.05$). To address statistical constraints associated with analyzing proportions and near-zero values, a constant value ($1x10^{-6}$) was added to all proportions of items eaten.

As carabids may consume food items in captivity that are not consumed in nature (Larochelle 1990), a *Likelihood of Consumption Index* (LOCI) was constructed to enable conservative prediction of consumption potential in the field. Calculation for the index differs from that of previously utilized formulae for interpreting consumption (Waldbauer 1968, Sunderland and Vickerman 1980, Oberholzer and Frank 2003, Rutledge et al. 2004). The LOCI is defined here as the proportion of beetles consuming an item (frequency) multiplied by the proportion of item(s) consumed (proportion eaten). As a product of two proportions the result is bound between zero and one. This product results in up-weighting for items consumed in high frequency and quantity, and down-weighting for items consumed rarely and/or in small quantity. The LOCI adjustment thus dampens outliers for cases with dissimilar combinations of frequency and consumption. This index assumes beetles were hungry and test item discovery occurred, resulting in either

acceptance or rejection. To apply a general label of either eaten or not, a minimum LOCI threshold value of 0.10 was used. For example, a value of 0.10 may translate to the case where 50% of beetles consumed one (20%) of the items presented. The results of applying this subjective threshold are consistent with observations of test item probing as opposed to sustained feeding behavior.

To examine size-dependent feeding preferences by *E. xanthopus*, the relationship between test item size (volume) and likelihood of consumption was examined by regressing consumption index values (above 0.10) against the ellipsoid biovolume (Kuschka 1994) of each test item (Table 2.1) using linear regression (PROC REG; SAS Institute Inc. 2008). *Elaphropus xanthopus* ellipsoid biovolume (≈0.71 mm^3) was retrieved from species estimates (Appendix G) and overlaid on the regression plot. All biovolume data was natural log transformed prior to analysis.

Choice tests

For the pollen-only choice test, differences in consumption of Bt versus non-Bt pollen cakes were examined using a paired t-test (PROC TTEST; SAS Institute Inc. 2008). For the frequency-based choice test, observed consumption as it deviated from expected consumption (based on initial frequencies) was evaluated as relative risk (θ) (*after* Weale et al. 2000, Shigemiya 2004):

$$\theta = \ln[R_E/R_P]$$

where R_E is the consumption of eggs (number of eggs [N_E] eaten/N_E provided) and R_P is the consumption of pollen cakes (number of cakes [N_P] eaten/N_P provided). Data was censored for cases where all test items or no test items were consumed, as choice may not be determined. Frequency of food availability (N_E/N_P) was natural log transformed prior to analysis. Risk (θ), hereafter called preference, was regressed against natural log transformed frequencies to assess the influence of alternative food availability on corn pollen consumption (PROC REG; SAS Institute Inc. 2008).

Results

No-choice tests

The proportion of items eaten by *E. xanthopus* in no-choice tests varied markedly (F = 82.92; df = 33, 580; $P < 0.001$). Examining consumption through the adjustment made by the LOCI (Table 2.2) revealed that small eggs (e.g. *D. melanogaster*, collembola, soil mites), and early juvenile stages of arthropods (e.g. second instar *D. melanogaster*), were more readily consumed over larger eggs (e.g. SCRW and WCRW eggs) and later juvenile stages (e.g. third instar *D. melanogaster*). Consumption of small test items in a dead state was also more frequent for specific treatments. Of the plant items tested, each was either negligibly or not consumed. Regression of LOCI values against test item volume (Figure 2.2) revealed a significant trend for decreasing likelihood of consumption by adult *E. xanthopus* as test item size increased (F = 15.86; df = 1, 19 ; P = 0.001; R^2 = 0.455). The majority of test items consumed were smaller in volume than *E. xanthopus*, and between 0.05 and 0.55 mm in width (Table 2.2); the maximum mandibular gape of *E. xanthopus* is ≈0.30 mm (Appendix K).

Choice tests

When presented a 1:1 of Bt and non-Bt corn pollen, consumption by *E. xanthopus* did not differ ($t = 0.52$; df = 16; $P = 0.607$). Adults consumed a mean (± SE) percentage of 44 ± 11% Bt pollen cakes and 51 ± 11% non-Bt pollen cakes (n=17); together amounting to ≈700 grains over the 4 h test period. Since *E. xanthopus* did not distinguish between Bt and non-Bt pollen, preference values were pooled over both pollen types for the frequency regression. There was no influence of food type frequency on *E. xanthopus* preference ($F = 0.22$; df = 1, 56; $P = 0.640$). As *E. xanthopus* exhibited frequency-independent consumption, preference for fly eggs over pollen was approximated by the y-intercept ($\beta_0 = 2.79$) (Figure 2.3). An intercept preference value (θ) of 2.79 translates into the average case where all fly eggs were eaten, and ≈6% (0.0613) of each available pollen cake was eaten ($\ln[\theta] = 16.3 = 1/0.0613$). For the average case of 3 available pollen cakes, 0.184 pollen cakes were eaten (≈44 pollen grains) over the 4 h test period.

Discussion

The main objective of this study was to broadly estimate the diet breadth of a ground beetle species, *Elaphropus xanthopus*, in order to predict its usefulness as a bioindicator species in nontarget risk assessment programs. Choice and no-choice tests identified corn pollen and immature stages of microarthropods as potential direct and indirect exposure routes for transgenic toxins, respectively. The findings here also narrow the number of likely direct and indirect pathways through which adult *E. xanthopus* may be exposed to gut-active transgenic toxins, as neither corn anthers were consumed nor were potential

bioaccumulator organisms (e.g. collembola, soil mites; Bitzer et al. 2005, Griffiths et al. 2006).

In choice tests, *E. xanthopus* did not distinguish between Cry34/35Ab1 or non-Bt corn pollen. Corn pollen consumption and non-preference suggests *E. xanthopus* is capable of direct exposure to transgenic toxins during anthesis in the cropping environment. In cases where fields are monocropped with rootworm-resistant plants, or corn stands include mixtures of rootworm-resistant and non-Bt plants (e.g. refuge-in-the-bag), *E. xanthopus* may be expected to consume transgenic corn pollen in relative proportion to its availability. The presence of preferred food items, however, may strongly attenuate toxin exposure via pollen, as fly eggs were consumed independent of corn pollen availability (Figure 2.3). In the absence of alternative food, approximately 48% (≈700 grains) of available pollen was consumed in a single meal, whereas in the presence of fly eggs, pollen consumption was reduced to approximately 6% (≈44 grains). The question of lethal or sub-lethal toxicity given Bt pollen exposure remains, although a non-controlled study found adult carabids may be sustained for extended periods under *ad libitum* feeding (Mullin et al. 2005). To address these questions, further studies quantify *E. xanthopus* exposure to rootworm-resistant proteins in the laboratory and field (Chapter 3), and subsequently examine the consequences of chronic Bt pollen exposure (Chapter 4).

Elaphropus xanthopus adults consumed ten of the fifteen species examined (Table 2.2), where consumption was heavily dependent upon test item life-stage, physical state (e.g.

dead, alive) (Table 2.1), and size (Figure 2.2). *Elaphropus xanthopus* readily consumed eggs and early juvenile stages of arthropods, as well as dead microarthropods. The most consumed items included eggs and larvae of *Drosophila melanogaster* (Dm), nematode adults, eggs of collembola and soil mites, and first instar southern and western corn rootworms (*Diabrotica* spp.). For other small-bodied carabid beetles, fly eggs (Finch 1996) and larvae (Hering 1998) are generally expected to be part of the diet, and collembola are thought to be a key food resource (Hengeveld 1980a/b). In addition to collembola, highly abundant soil mites fall within the size range of prey that *E. xanthopus* may take (Figure 2.2). Living predatory and soil mites were not consumed in the present study, confirming nighttime field observations using infra-red video equipment, where an *E. xanthopus* adult was observed to encounter, investigate, then disregard a soil mite adult (M.D. Lepping, unpubl. data). While mites were not eaten alive in the no-choice tests, dead predatory mites were consumed. Consumption of dead mites and collembola suggest *E. xanthopus* scavenges opportunistically. Consumption of nematodes, and eggs of collembola, flies, and soil mites by adult *E. xanthopus* suggest the ability to locate and consume very small prey, and the potential for these abundant taxa to serve as sustainable food resources.

Consistent with trends for large ratios of predator-to-prey body size within terrestrial invertebrates (Warren and Lawton 1987), *E. xanthopus* is considerably larger than test items which attained the highest LOCI values (Figure 2.2). For example, smaller (immature stage) test items of the same species (*D. melanogaster*) were consumed in greater number, as would be expected relative to beetle body volume and maximum

mandibular gape. Several test items that were not consumed were also within the range of prey body sizes acceptable to *E. xanthopus*. Negligible or zero consumption of specific test items may reflect non-preference or rejection of those potential prey items in the field.

Small seeds of common chickweed species were probed and consumed in very small quantities by *E. xanthopus*, while larger seeds were not. Seeds are accepted by some predaceous *Bembidion* species, although preference for seed is likely determined by seed size and hardness, and not necessarily nutritional composition (Goldschmidt and Toft 1997). Based on the plant species tested, it is unlikely that *E. xanthopus* consumes seeds under field conditions.

In terms of biological control potential for ground-dwelling pests, adult *E. xanthopus* exhibited zero consumption of slug life-stages, moderate consumption of rootworm larvae (LOCI ≈0.5) and eggs (LOCI ≈0.2), and high consumption of fly egg stages (Table 2.2). While some larger bodied carabids exhibit preference for certain species of slug eggs (Oberholzer and Frank 2003), some probing or investigative feeding would have been expected if slug tissue comprised part of the diet for adult *E. xanthopus*. In Maryland, *E. xanthopus* adult abundance is highest in July when corn anthesis occurs (Chapter 1), coinciding with western corn rootworm (WCRW) adult eclosion and oviposition (Pierce and Gray 2006). Early clutches of WCRW eggs may be oviposited in soil cracks at the base of corn plants (Foster 1979, Tollefson and Calvin 1994) – a microhabitat that small, abundant predators such as *E. xanthopus* inhabit (Dively et al. in

prep.). This study used *D. melanogaster* (Drosophilidae) as a surrogate for fly pests in the family Anthomyiidae, including cabbage root fly (*Delia radicum* L.), onion maggot (*Delia antiqua* Meigen), and seedcorn maggot (*Delia platura* Meigen). Although anthomyiid fly eggs and larvae are larger than *D. melanogaster*, predation would be expected to occur for cases where fly eggs are accessible, as *E. xanthopus* readily consumed all active immature stages of *D. melanogaster*. These findings suggest *E. xanthopus* adults may contribute to biological control in epigeal habitats for crop pests in early immature stages.

Eggs from foliar-feeding agricultural pests were included in this study to observe consumption of eggs varying in size and structure. Within this egg group, *E. xanthopus* consumed a moderate amount of black cutworm (BCW) eggs, few European corn borer (ECB) egg masses, and no Colorado potato beetle (CPB) eggs. While several carabid species may forage on plant foliage [carnivores: *Agonum dorsale* (Sunderland and Vickerman 1980); predaceous parasitoids: *Lebia grandis* (Weber et al. 2008); seed predators: several species (Saska 2005, Honěk et al. 2007)], *E. xanthopus* has only been observed to forage on the ground (M.D. Lepping, pers. obs.), and is therefore not likely an egg predator of the foliar-feeding species tested.

Within the scope of the test item set, an apparent preference for selective animal material by *E. xanthopus* suggests this species is primarily carnivorous and may include saprophagy. Moreover, the mandible morphology of *E. xanthopus* suggests a trophic identity more closely aligned to carnivorous carabids (e.g. long terebral ridge of incisor

(shearing surface), high length-to-width ratio for mandibles as compared to more omnivorous ones (*sensu lato* granivorous) (Frank 2007). Concurrently, opportunistic consumption of corn pollen by *E. xanthopus* is expected in the field, although this prediction remains to be tested through gut dissections and molecular probing. Nonetheless, corn pollen feeding in the laboratory by adult *E. xanthopus*, coupled with high abundance in no-till field corn systems (Chapter 1), presents the opportunity for direct feeding exposure to transgenic toxins. If the diet breadth of this carabid species is narrowed to egg predation and scavenging, and further limited by size restrictions, then exposure to transgenic toxins by way of pollen is realistic relative to other potential food items, and may be further explored to adequately characterize this exposure route (Chapter 3) and the potential for adverse effects (Chapter 4).

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Table 2.1. No-choice feeding test items presented to *Elaphropus xanthopus* (Dejean) adults.

Common Name	Species	Food Test Item (quantity presented)	State	n	Mean length ±SE (mm)	Mean width ±SE (mm)	≈Vol. (mm^3)
bluegrass	*Poa pratensis* L.	bluegrass seeds (5)	viable	20	2.80 ± 0.11	0.79 ± 0.02	0.93
common chickweed	*Stellaria media* (L.) Villars	common chickweed seeds (5)	viable	20	1.00 ± 0.07	0.84 ± 0.03	0.26
lesser chickweed	*Stellaria media* ssp. *pallida* (Dumortier) Ascherson & Graebner	lesser chickweed seeds (5)	viable	20	0.55 ± 0.02	0.47 ± 0.01	0.041
field corn	*Zea mays* L.	non-Bt corn anthers (5)	—	20	5.33 ± 0.27	1.22 ± 0.09	1.7
"	*Zea mays* L. + Cry34/35Ab1	Cry34/35Ab1 corn anthers (5)	—	20	5.40 ± 0.10	1.28 ± 0.07	1.8
slug	*Deroceras* sp.	slug eggs (5)	viable	3	2.20 ± 0.11	1.69 ± 0.04	3.3
"	"	slug adult (1)	alive	20	≈20	≈10	1000
"	"	"	dead	20	"	"	"
roundworm	*Caenorhabditis briggsae*	nematode adults (5)	alive	20	0.67 ± 0.11	0.04 ± 0.001	5.6×10^{-4}
soil mite	*Pergalumna corrugis* (Jacot)	soil mite eggs (5)	viable	20	0.24 ± 0.004	0.13 ± 0.01	0.0021
"	"	soil mite adults (5)	alive	20	0.73 ± 0.04	0.56 ± 0.01	0.12
predatory mite	*Parasitus* sp.	predatory mite adults (5)	alive	2	0.87 ± 0.08	0.42 ± 0.02	0.057
"	"	"	dead	7	"	"	"
springtail	*Entomobrya intermedia* Brook	collembolan eggs (5)	viable	20	0.16	0.15	0.0018
"	"	collembolan eggs (5 clusters of 4)	viable	20	—	—	—
"	"	1^{st} instar collembola (5)	alive	9	0.39 ± 0.01	0.11 ± 0.01	0.0025
"	"	"	dead	9	"	"	"
"	"	collembola adults (5)	alive	20	1.94 ± 0.22	0.35 ± 0.02	0.11
"	"	"	dead	20	"	"	"

Table 2.1. No-choice feeding test items presented to *Elaphropus xanthopus* (Dejean) adults, Continued.

Common Name	Species	Food Test Item (quantity presented)	State	n	Mean length ±SE (mm)	Mean width ±SE (mm)	≈Vol. (mm^3)
Colorado potato beetle (CPB)	*Leptinotarsa decemlineata* (Say)	CPB eggs (5)	viable	20	≈1.8	≈0.8	0.60
Southern corn rootworm (SCRW)	*Diabrotica undecimpunctata howardi* Barber	SCRW eggs (5)	viable	40	0.66 ± 0.01	0.42 ± 0.01	0.061
"	"	SCRW 1st instar larvae (5)	alive	15	1.1	0.17	0.017
Western corn rootworm (WCRW)	*Diabrotica virgifera virgifera* LeConte	WCRW eggs (5)	viable	40	0.64 ± 0.04	0.47 ± 0.03	0.074
"	"	WCRW 1st instar larvae (5)	alive	18	1.34 ± 0.14	0.24 ± 0.02	0.04
Black cutworm (BCW)	*Agrotis ipsilon* (Hufnagel)	BCW eggs (5)	viable	20	0.54 ± 0.01	0.44 ± 0.01	0.063
"	"	BCW 1st instar larvae (5)	alive	20	3.1 ± 0.1	0.34 ± 0.02	0.19
"	"	BCW 3rd instar larvae (5)	alive	20	7.0 ± 0.3	0.70 ± 0.01	1.8
European corn borer (ECB)	*Ostrinia nubilalis* (Hübner)	ECB egg masses (5 clusters of ≈8)	viable	20	—	—	—
thief ant	*Solenopsis (Diplorhoptrum)* sp.	thief ant adults (5)	dead	10	1.65 ± 0.18	0.26 ± 0.01	0.058
vinegar fly (Dm)	*Drosophila melanogaster* Meigen	Dm eggs (5)	viable	20	0.50 ± 0.01	0.20 ± 0.01	0.010
"	"	Dm 2nd instar larvae (5)	alive	20	2.41 ± 0.16	0.47± 0.05	0.28
"	"	"	dead	20	"	"	"
"	"	Dm 3rd instar larvae (5)	alive	20	3.73 ± 0.26	0.82 ± 0.07	1.3
"	"	"	dead	20	"	"	"
"	"	Dm pupae (5)	alive	20	2.25 ± 0.13	1.00 ± 0.02	1.2

Table 2.2. Consumption of no-choice feeding test items by *Elaphropus xanthopus* (Dejean) adults, ranked by Likelihood of Consumption Index (LOCI) values.

LOCI ≥ 0.10[a]	Test Item (quantity presented)[b]	State	n	Proportion of beetles that ate[c]	Mean ± SE eaten[d]	Proportion eaten[e]	Group[f]	Likelihood of Consumption Index (LOCI)
Yes	Dm eggs (5)	viable	20	1	4.8 ± 0.2	0.96 ± 0.03	A	0.96
Yes	nematode adults (5)	alive	20	1	4.7 ± 0.2	0.94 ± 0.03	A	0.94
Yes	collembolan eggs (5 clusters of 4)	viable	20	1	18.3 ± 0.2	0.91 ± 0.03	A	0.91
Yes	collembolan eggs (5)	viable	20	1	4.1 ± 0.2	0.81 ± 0.03	AB	0.81
Yes	Dm 2nd instar larvae (5)	dead	20	1	3.5 ± 0.2	0.70 ± 0.03	BC	0.7
Yes	Dm 2nd instar larvae (5)	alive	20	0.95	3.6 ± 0.2	0.69 ± 0.03	BC	0.66
Yes	SCRW 1st instar larvae (5)	alive	15	1	2.7 ± 0.2	0.54 ± 0.04	CD	0.54
Yes	WCRW 1st instar larvae (5)	alive	18	0.94	2.6 ± 0.2	0.49 ± 0.04	DE	0.46
Yes	soil mite eggs (5)	viable	20	0.85	3.2 ± 0.2	0.54 ± 0.03	CD	0.46
Yes	collembola adults (5)	dead	20	0.9	2.5 ± 0.2	0.44 ± 0.03	DE	0.40
Yes	BCW eggs (5)	viable	20	0.95	2.0 ± 0.2	0.38 ± 0.03	DEF	0.36
Yes	1st instar collembola (5)	dead	9	0.89	1.8 ± 0.4	0.36 ± 0.05	DEFG	0.32
Yes	BCW 1st instar larvae (5)	alive	20	0.85	1.8 ± 0.2	0.31 ± 0.03	EFG	0.26
Yes	SCRW eggs (5)	viable	40	0.88	1.6 ± 0.2	0.28 ± 0.02	FG	0.25
Yes	predatory mite adults (5)	dead	7	0.71	1.6 ± 0.4	0.22 ± 0.06	EFGHI	0.16
Yes	WCRW eggs (5)	viable	40	0.63	1.6 ± 0.2	0.21 ± 0.02	GH	0.13
Yes	Dm 3rd instar larvae (5)	alive	20	0.8	1.0 ± 0.2	0.16 ± 0.03	GHI	0.13
Yes	ECB egg masses (5 clusters of ≈ 8)	viable	20	0.95	0.5 ± 0.2 (≈ 4 eggs)	0.10 ± 0.03	HI	0.10

Table 2.2. Consumption of no-choice feeding test items by *Elaphropus xanthopus* (Dejean) adults, ranked by Likelihood of Consumption Index (LOCI) values, Continued.

LOCI ≥ 0.10[a]	Test Item (quantity presented)[b]	State	n	Proportion of beetles that ate[c]	Mean ± SE eaten[d]	Proportion eaten[e]	Group[f]	Likelihood of Consumption Index (LOCI)
No	lesser chickweed seeds (5)	viable	20	0.4	0.6 ± 0.3	0.05 ± 0.03	HI	0.02
No	Dm 3rd instar larvae (5)	dead	20	0.15	1.2 ± 0.5	0.04 ± 0.03	I	0.01
No	CPB eggs (5)	viable	20	0.05	0.4 ± 0.9	0.01 ± 0.03	I	< 0.01
No	common chickweed seeds (5)	viable	20	0.05	0.1 ± 0.9	0.001 ± 0.03	I	< 0.01
No	non-Bt corn anthers (5)	—	20	0	0	0	I	0
No	Cry34/35Ab1 corn anthers (5)	—	20	0	0	0	I	0
No	bluegrass seeds (5)	viable	20	0	0	0	I	0
No	slug eggs (5)	viable	3	0	0	0	I	0
No	slug adult (1)	alive	20	0	0	0	I	0
No	slug adult (1)	dead	20	0	0	0	I	0
No	soil mite adults (5)	alive	20	0	0	0	I	0
No	predatory mite adults (5)	alive	2	0	0	0	—	0
No	1st instar collembola (5)	alive	9	0	0	0	I	0
No	collembola adults (5)	alive	20	0	0	0	I	0
No	BCW 3rd instar larvae (5)	alive	20	0	0	0	I	0
No	thief ant adults (5)	dead	10	0	0	0	I	0
No	Dm pupae (5)	alive	20	0	0	0	I	0

[a] hatched line defines a threshold of 0.10 for the LOCI (see Materials and Methods).

[b] for acronym definitions, see Table 2.1 (Materials and Methods).

[c] sum of beetles that consumed some portion of any item presented divided by n (total beetles presented test items).

[d] the average consumption of test items presented when at least some portion of any test item was consumed.

[e] the mean eaten divided by the number of items presented (± SE).

[f] group membership assignment for the proportion of test item(s) consumed;
means followed by the same letter within column were not statistically different (Tukey-Kramer test; $\alpha = 0.05$).

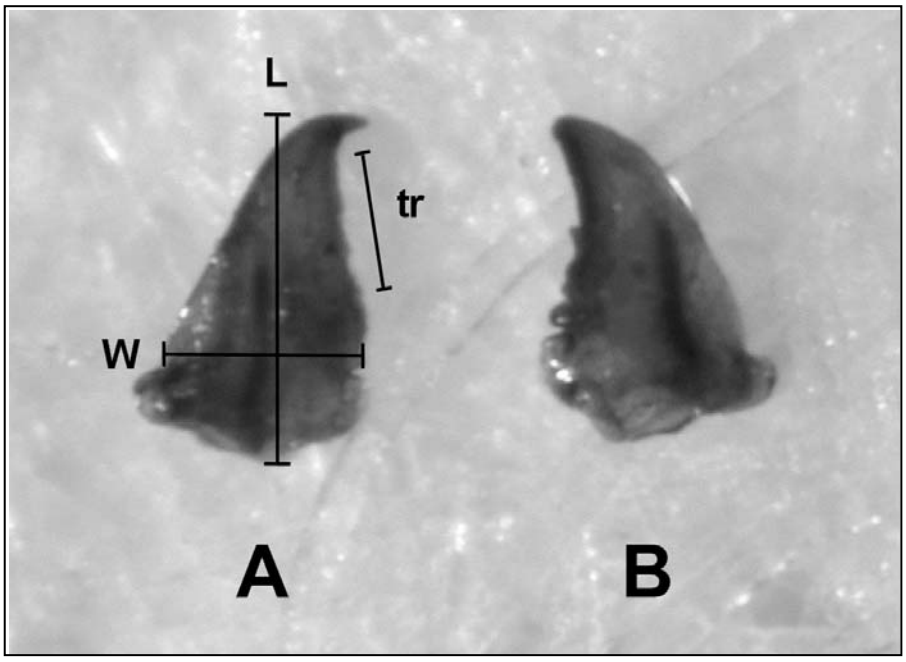

Figure 2.1. Left (A) and right (B) mandibles of adult *Elaphropus xanthopus* (Dejean), dorsal aspect. Abbreviations: L, mandible length; W, mandible width; tr, terebral ridge (*after* Acorn and Ball 1991).

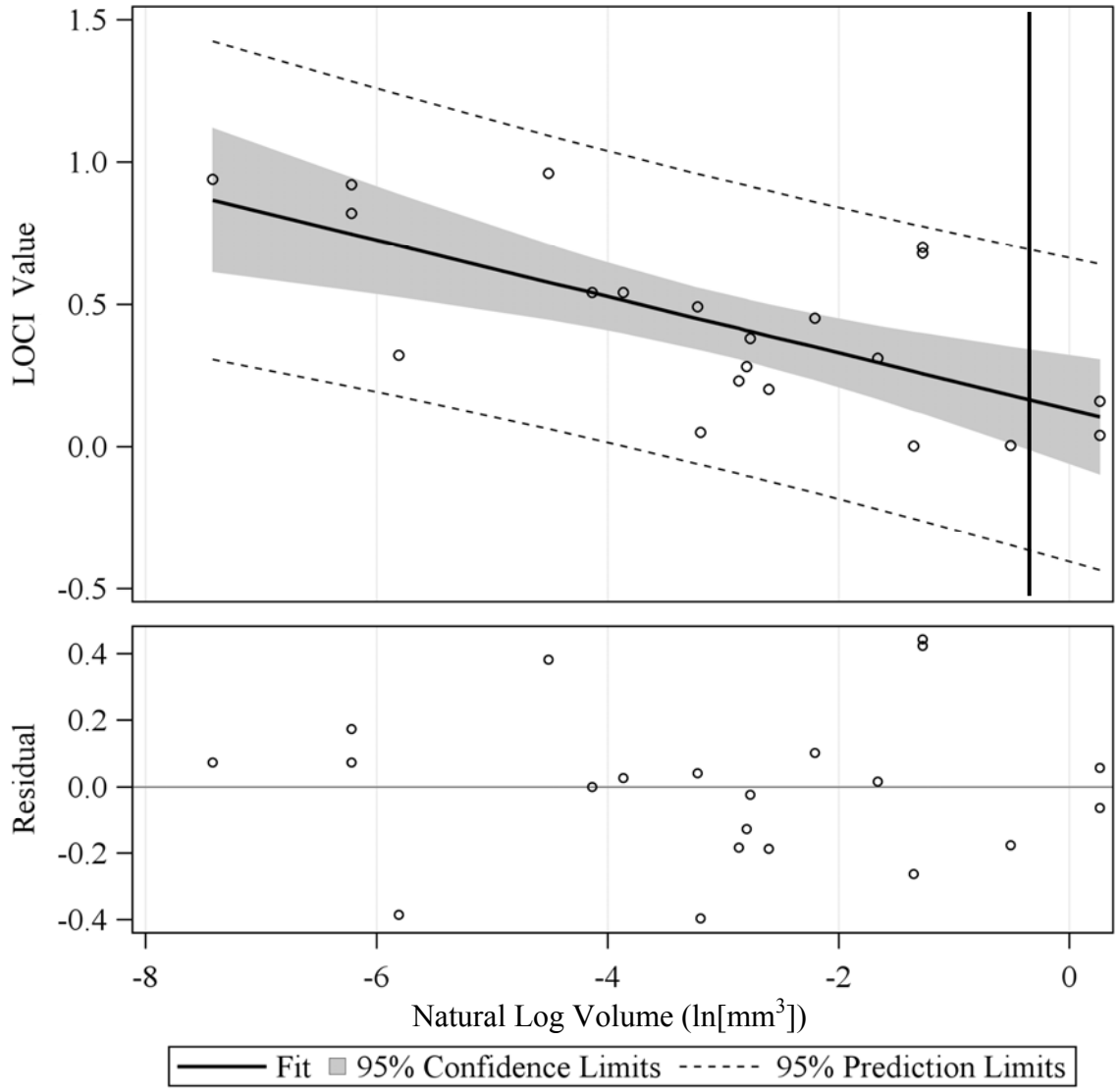

Figure 2.2. Relationship between *Likelihood of Consumption Index* (LOCI) values for adult *Elaphropus xanthopus* (Dejean) and test item volume (natural log transformed). *Likelihood of Consumption* is calculated as the proportion of beetles accepting test items multiplied by the proportion of test items consumed in no-choice tests. Test item volume is estimated as an ellipsoid (π/6*length*width*height) (*after* Kuschka 1994). Plotted data includes only observations for which consumption was recorded. The relationship is described by the model *Likelihood of Consumption* = 0.130 - 0.099*ln(test item volume); R^2 = 0.4550, n = 21, root mean square error = 0.2381, P = 0.001. The vertical solid line represents the approximate ellipsoid body volume of adult *E. xanthopus* (ln[mm^3]).

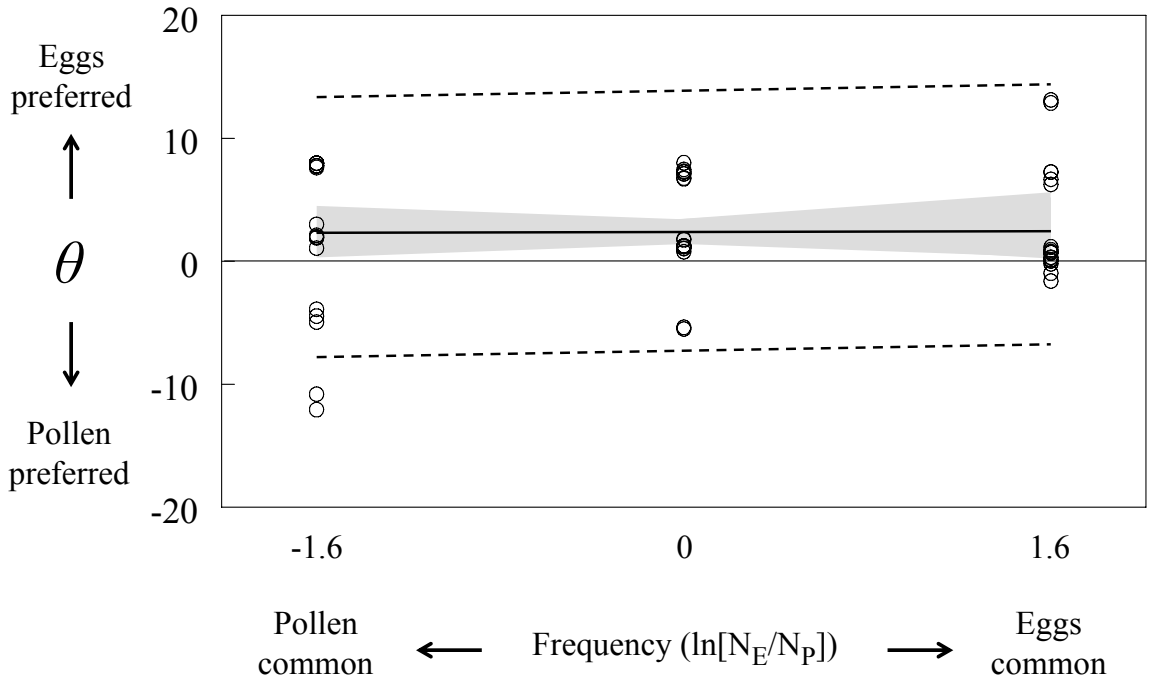

Figure 2.3. Relationship between food availability (Frequency) and preference (θ) for *Elaphropus xanthopus* (Dejean) adults. Three ratios of corn pollen cakes (N_P) to *Drosophila melanogaster* (Meigen) eggs (N_E) were presented (5:1, 1:1, 1:5). The relationship is described by the model $\theta = 2.79 + 0.24*\ln(N_E/N_P)$; $R^2 = 0.004$, n = 58, root mean square error = 5.132, $P = 0.640$. Shaded area contains the 95% confidence limits for the coefficient; hatched lines define the 95% prediction limits. Note: *E. xanthopus* exhibited frequency-independent consumption, allowing preference to be estimated by the y-intercept.

CHAPTER 3: Nontarget exposure to rootworm-resistant transgenic proteins: laboratory and field uptake of Cry34Ab1 by a ground beetle, *Elaphropus xanthopus* (Coleoptera: Carabidae)

Abstract

A laboratory and field study determined the half-life of detectability and estimated field exposure to Cry34Ab1 Bt corn protein for a nontarget ground beetle, *Elaphropus xanthopus* (Dejean) (Coleoptera: Carabidae). Using quantitative enzyme-linked immunosorbent assay (ELISA), whole-body adult ground beetles were screened for the presence and quantity of Cry34Ab1 protein, which largely predicts toxicity for the target pest, corn rootworm (*Diabrotica* spp.). Laboratory half-life studies estimated Cry34Ab1 protein remaining in beetles following consumption of a single Bt pollen meal at post-consumption time periods ranging from 0 to 96 h. The half-life of detectability for pollen-borne Cry34Ab1 in *E. xanthopus* was approximately 11 h, with detectability ending after 36 h. To estimate laboratory dosing, *E. xanthopus* frass produced during long-term Bt pollen-only feeding was examined for Cry34Ab1 protein. *Elaphropus xanthopus* frass yielded approximately 37% of the Cry34Ab1 protein found in Cry34/35Ab1 pollen, indicating organism-level exposure occurred. In field experiments, *E. xanthopus* were collected during anthesis from both Cry34/35Ab1 and non-Bt field corn plots. Of *E. xanthopus* collected from field plots planted with Cry34/35Ab1 field corn, 57% tested positive for the presence of Cry34Ab1 (11.0 ± 1.7 ng mg^{-1}), whereas 32% tested positive in non-Bt (3.0 ± 1.7 ng mg^{-1}) corn plots approximately 40 m away. While field results underscore the need for larger test-plot size in community studies examining transgenic

toxins, Cry34Ab1 concentrations found within field exposed *E. xanthopus* were below laboratory doses found to cause no effects on adult performance in related long-term studies.

Key Words: *Bacillus thuringiensis*, enzyme-linked immunosorbent assay (ELISA), nontarget organisms, risk assessment

Introduction

One of the major advantages of plant-incorporated transgenic toxins based on *Bacillus thuringiensis* (Bt) is the host (target) specificity they exhibit, often narrowed to taxonomic levels lower than the order-level specificity reflected in some bacterial Bt strains (Höfte and Whiteley 1989). However, relatively few nontarget species have been examined for Bt protein exposure despite the large scale deployment of Bt crops (USDA NASS 2009). Intoxication via Bt exposure must occur through oral consumption, as toxin binding receptors are found along the mid-gut lining (Schnepf et al. 1998). Exposure routes (pathways) through which nontargets may be exposed to transgenic Bt proteins in the cropping environment include direct and indirect pathways. Direct exposure is hypothesized to occur through feeding on plant tissues or via uptake of exudates produced during plant senescence. Indirect exposure may occur through consumption of intoxicated, bioaccumulator, or contaminated prey.

Currently, four different Bt proteins (Cry3Bb1, Cry34/35Ab1, mCry3A) are expressed in EPA-registered corn hybrids to control root-feeding beetle pests in the genus, *Diabrotica* (Coleoptera: Chrysomelidae), a billion dollar pest complex that necessitates pest management options in field corn (Metcalf 1986, Gray 2000). As diabroticine rootworms expand their geographic range through natural and anthropogenic processes, increased Bt adoption is expected. With increased Bt crop deployment, nontarget coleopteran exposure to rootworm-resistant Bt proteins may be likely during crop phenological events (e.g. anthesis, senescence, harvest) in which plant tissues become readily available for direct

or indirect uptake. Anthesis as a Bt exposure event has been reported for non-target Araneae (Ludy and Lang 2006), Coleoptera (Lundgren et al. 2005, Harwood et al. 2007, Álvarez-Alfageme et al. 2009), Heteroptera, Neuroptera (Obrist et al. 2006), and Lepidoptera (Oberhauser et al. 2001, Pleasants et al. 2001). Corn pollen is a relatively large, wind-dispersed pollen, most of which is deposited within the site of origin (Raynor et al. 1972). During peak anthesis, pollen grain density within a field may exceed 500 grains cm^{-2} (Smith et al. 2004), and further exceed 1000 grains cm^{-2} at ground level (M.D. Lepping, unpubl. data). Nontarget coccinellids that consume corn pollen directly from plants (Cottrell and Yeargan 1988, Lundgren et al. 2004) have received focus in Bt risk assessment programs (Cry1Ab: Bai et al. 2005; Cry3Bb/1: Lundgren and Wiedenmann 2002, Ahmad et al. 2006). In the epigeal environment, ground-dwelling beetle species may also be exposed to Bt proteins by way of direct pollen consumption (Álvarez-Alfageme et al. 2009).

Ground beetles in the family Carabidae are considered beneficial organisms as they may consume crop pests (Kromp 1999). In arable systems, carabid assemblages are speciose and abundant, and thus may be subject to nontarget effects imposed by rootworm-resistant transgenic toxins. Previous studies have found carabid exposure to Cry protein residues in transgenic corn systems (Zwahlen and Andow 2005, Álvarez-Alfageme et al. 2009, Peterson et al. 2009), though exposure patterns based on body size or trophic affiliation are unclear. For omnivorous species, ≈50% of *Harpalus pensylvanicus* (large-bodied) and *Clivina bipustulata* (small-bodied) tested positive for Cry1Ab (lepidopteran-active), whereas only 4% of *Stenolophus comma* (small-bodied) tested positive in a Bt

corn system (Peterson et al. 2009). For large-bodied carnivores, detection of Bt exposure has been absent (*Scarites subterraneus*; Harwood et al. 2006, Peterson et al. 2009), low (8%, *Poecilus cupreus*; Álvarez-Alfageme et al. 2009), and high (50+% for *Cyclotrachelus iowensis*, *Poecilus chalcites*, *P. lucublandus*; Zwahlen and Andow 2005). Few studies have examined Bt exposure for small-bodied carabids (Peterson et al. 2009) and fewer have included small-bodied carnivores (Zwahlen and Andow 2005). Direct corn pollen consumption by several carabid species in captivity (Mullin et al. 2005, Chapter 2), and the likelihood of smaller bodied carabids consuming pollen in the epigeal environment may combine to increase exposure and the potential for toxicity.

Viewing anthesis as a realistic exposure event for carabid taxa (Álvarez-Alfageme et al. 2009), the present study aimed to estimate exposure to Bt protein by *Elaphropus xanthopus* (Dejean 1831) (Coleoptera: Carabidae), a small-bodied (≈2 mm length), micro-invertebrate predator (Chapter 2) distributed throughout most of eastern North America (Bosquet and Larochelle 1993). Adult *E. xanthopus* are found in moderate to high densities in Maryland corn systems (Chapter 1), where newly eclosed adult populations coincide with corn anthesis. Previous laboratory studies found adult *E. xanthopus* to consume corn pollen but not corn anthers (Chapter 2), both of which are deposited in the epigeal environment during anthesis. This carabid species satisfied criteria commonly sought when identifying potential biological indicators, including feasibility of field monitoring (e.g. wide distribution, high abundance in field corn), co-occurrence with potential exposure events (Chapter 1), carnivorous tendencies and morphology, and the potential for corn pollen feeding in the field (Chapter 2).

Using enzyme-linked immunosorbent assay (ELISA) as a screening method, studies included three components: (1) examination of the half-life of detectability and decay rate of Cry34Ab1 following *E. xanthopus* consumption of Cry34/35Ab1 pollen, in order to predict the likelihood of detection in the field given limited exposure; (2) estimation of organism-level Cry34Ab1 exposure in the laboratory (dosing), using protein consumption data from half-life studies (input), and protein concentration values from frass material (output); and (3) estimation of Cry34Ab1 concentrations from *E. xanthopus* collected in Bt and non-Bt field plots. From previously reported values of Cry34/35Ab1 protein content in pollen (US EPA 2005), Cry35Ab1 levels were expected to be low or undetectable. Therefore, samples were assayed to estimate Cry34Ab1 concentrations only, which largely predict toxicity for the target pest, corn rootworm (*Diabrotica* spp.) (Herman et al. 2002).

Materials and Methods

Beetle collection and maintenance

Elaphropus xanthopus adults were collected from field sites at the U.S. Department of Agriculture (USDA), Beltsville, MD. Collection was conducted in 0.11 ha plots established with either Cry34/35Ab1 field corn or its non-Bt near isoline (Dow AgroSciences, Indianapolis, IN). To reduce the likelihood of pre-experiment exposure to Bt plant tissues, separate equipment was used to collect beetles in Bt and non-Bt plots. Specimen collection was accomplished by vacuuming soil and detritus surrounding corn plants. Vacuum samples were emptied into collection trays and *E. xanthopus* adults were

aspirated into storage vials containing moist filter paper. For half-life studies, vials containing *E. xanthopus* were placed in coolers and transported to the laboratory, where beetles were maintained for one month prior to experiment in a growth chamber at 20°C and a 16:8 (light:dark) cycle. Beetles were fed ground, moistened dog food (IAMS Smart Puppy; IAMS Co., Dayton, OH) every 5 d. Two days prior to experiment, beetles were allowed to feed for 24 h, and then were starved for 24 h. For field exposure studies, vials containing beetles were transferred from the field packed in dry ice; then stored at -20°C upon returning to the laboratory.

Pollen collection and storage

Cry34/35Ab1 corn pollen (mean (± SE) of 34.2 ± 1.1 ng mg^{-1} Cry34Ab1 dry weight) was collected from field plots at the University of Maryland's Central Maryland Research and Education Center (CMREC), Beltsville, MD. Pollen was collected directly from plants by shaking tassels inside large paper bags during peak anthesis. Pollen was then transported to the laboratory, meshed to remove anthers and other contaminants, and stored at -20°C.

Cry34Ab1 detectability and decay experiment

After starvation, individual beetles from non-Bt field plots were placed in Petri dishes (50 x 9 mm; 351006, BD Falcon™ Tight-fit Lid Dish, Becton Dickenson Biosciences Discovery Labware, Bedford, MA) containing filter paper and a single pollen cake. Dishes were kept upside down and dry during loading to prevent pollen accessibility prior to experiment. Pollen cakes were formed by pipetting a 5 µL aliquot of Cry34/35Ab1 pollen from 0.40 g mL^{-1} dH_2O stock suspensions on to 70 mm diameter

filter paper (porosity 8; Fisher Scientific Co., Pittsburg, PA). All dishes were then righted and supplied with dH_2O. At room temperature, beetles were allowed to consume a single pollen meal prior to removing individuals to allow for digestion. A pollen meal was defined as the combination of two events: (1) observation of sustained feeding behavior at the pollen cake; and (2) cessation of feeding behavior marked by subsequent movement away from the pollen cake. Individuals were monitored using a stereoscope to confirm feeding had occurred. After completing a pollen meal, each beetle was transferred singly to a microcentrifuge tube containing a mist of dH_2O, and stored at 25°C for a fixed period of digestion. Nine fixed digestion periods included (replication): 0 (28), 3 (33), 6 (25), 12 (26), 24 (19), 36 (26), 48 (36), 72 (17), and 96 h (17) following consumption. At the end of each digestion period, groups of beetles were stored at -20°C, until analysis.

Cry34Ab1 protein in E. xanthopus frass

Frass was obtained from ≈120 *E. xanthopus* adults feeding on Cry34/35Ab1 pollen in a related bioassay (toxicity) study (Chapter 4). To reduce the potential for contamination between frass and Cry34/35Ab1 pollen, frass was removed from Petri dishes where deposits were away from the pollen cake food source. All frass material was pooled into two samples (1.182 and 0.355 mg dry weight) and stored at -20°C. While this method did not allow for direct estimates of input and output for a single beetle, Cry34Ab1 concentrations for frass were directly compared to those for Cry34/35Ab1 pollen in this study, and related to values reported previously (US EPA 2005).

Cry34Ab1 protein in field collected E. xanthopus

Estimation of *E. xanthopus* exposure to Bt protein was conducted between 1000 and 1200 hrs during peak anthesis in Cry34/35Ab1 and non-Bt field corn plots. *Elaphropus xanthopus* adults were collected during a single sampling trip, 61 specimens from Cry34/35Ab1 plots and 60 specimens from non-Bt plots. Cry34/35Ab1 and non-Bt corn plots were separated by at least ≈40 m on all sides and neither plot was planted in Bt corn the previous year.

Immunoassay: sample preparation

Sample preparation for beetles (whole bodies), pollen, and frass was similar, except dilutions differed. Beetles used to determine Cry34Ab1 exposure in the field were washed in alternating 70% ethanol and dH_2O twice prior to extraction. Samples were homogenized with disposable tissue grinders (Kontes Pellet Pestles, Fisher Scientific Co., Pittsburg, PA) in 1.5 mL microcentrifuge tubes (Eppendorf, Westbury, NY), and diluted 1:2 (mg mL^{-1}) for beetles or 1:1 for frass and pollen in extraction buffer (Phosphate buffered saline with Tween®20, 2 mg mL^{-1} trypsin inhibitor Type II-S, pH 7.4; Sigma Aldrich, Saint Louis, MO). The mean whole body dry weight for field collected *E. xanthopus* adults (0.20 mg; Chapter 1) was substituted for actual fresh weight values in order to directly compare Bt protein concentrations on a dry weight-based basis across beetle, frass, and pollen samples. Homogenized samples were then vortexed for 30 s, and centrifuged at 8,000 rpm for 15 min. Supernatants were pipetted into 1.5 mL microcentrifuge tubes and stored at -20°C until immunoanalysis.

Immunoassay: ELISA screening

Sample extracts were thawed and vortexed for 30 s. Samples were diluted 2x, 5x, and 10x to determine if values for protein concentration were in the linear portion of the standard curve. Based on optimization findings, only 2x dilutions were analyzed for all samples. Immunological assay procedures followed manufacturer instructions (Beacon Analytical Systems, Portland, ME). Each ELISA plate included two negative control beetles, collected from field sites planted with either Cry34/35Ab1 or a non-Bt isoline, and subsequently maintained for one month prior to study. Standard curves were constructed for each plate using the following concentrations of purified Cry34Ab1 protein: 0, 0.3, 0.6, 1.2, 2.4, 3.6, 4.8, and 6.0 ng mL^{-1}. Standards were run in duplicate on each plate, and the resulting protein concentrations were averaged.

Immunoassay: quantification and analysis

Absorbance for each plate was read at 450 minus 605 nm using a microplate reader (SpectraMax Plus^{384}, Molecular Devices Corporation, Sunnyvale, CA), and standard curves were generated from the negative controls and Cry34Ab1 standards using associated software (SoftMax Pro, Molecular Devices Corporation, Sunnyvale, CA). Concentrations of Cry34Ab1 in sample extracts were determined by calculating the mean optical density reading against the standard curve. The threshold for positive detection was the mean Cry34Ab1 concentration of negative control beetles plus three standard deviations. Final Cry34Ab1 concentrations are reported as ng mg^{-1} (dry weight), accounting for dilution factors.

Statistical analysis

The detectability half-life of Cry34Ab1 was obtained by Log_{10} transforming the proportion of beetles testing positive for Cry34Ab1 at each period of digestion, then fitting transformed data using ordinary least squares linear regression of the form:

$$y_{(\text{Log}_{10}\text{ Proportion of Beetles Positive})} = b + ax,$$

where b is the y-intercept, a is decay rate, and x is period of time after feeding. Half-life of detectability was then calculated by solving for x when y = - 0.3010 (i.e. $\log_{10}$ of 0.5) (*after* Greenstone and Hunt 1993). To determine the decay rate of Cry34Ab1 protein following *E. xanthopus* consumption, mean protein concentrations were regressed against time after feeding (PROC REG; SAS Institute Inc. 2008), where a constant of one was added prior to natural log transformation of time.

To determine the effect of beetle digestion on Cry34Ab1 concentrations in frass as compared to those of undigested Cry34/35Ab1 pollen, and to examine the effect of collection site (field plot treatment) on Cry34Ab1 extracted from *E. xanthopus* adults, differences were examined using ANOVA (PROC MIXED; SAS Institute Inc. 2008).

Results

Cry34Ab1 detectability and decay experiment

Half-life of detectability for Cry34Ab1 following consumption of Cry34/35Ab1 pollen by *E. xanthopus* was ≈11 h (Figure 3.1). The longest detection period for Cry34Ab1 for the

same data was 36 h, with a projected maximum detection of 72 h after feeding (Figure 3.2). The mean (± SE) concentrations of Cry34Ab1 for beetles containing detectable amounts of Bt protein (≥0.3 ng mg^{-1}) ranged from 6.3 ± 0.9 at time 0 to 2.8 ± 0.5 at 36 h. For post-consumption periods greater than 36 h (48, 72, 96 h), no beetles tested positive for Cry34Ab1 presence.

Cry34Ab1 protein in E. xanthopus frass

Frass material from multiple *E. xanthopus* adults yielded a mean (± SE) of 12.6 ± 1.5 ng mg^{-1} Cry34Ab1, and differed significantly from Cry34/35Ab1 pollen (34.2 ± 1.1 ng mg^{-1} Cry34Ab1) ($F = 133.77$; df = 1, 4; $P = 0.0003$). Frass material contained 37% of the protein found in Cry34/35Ab1 pollen.

Cry34Ab1 protein in field collected E. xanthopus

Of *E. xanthopus* adults collected from a non-Bt corn plot, 32% tested positive (19 of 60 beetles) for Cry34Ab1 protein, where the mean (± SE) concentration was 3.0 ± 1.7 ng mg^{-1}. Of *E. xanthopus* adults collected from a Cry34/35Ab1 corn plot, 57% (35 of 61 beetles) tested positive for Cry34Ab1 protein, where the mean (± SE) concentration was 11.0 ± 1.7 ng mg^{-1}. For beetles testing positive in both non-Bt and Cry34/35Ab1 plots, mean protein concentrations between groups were significantly different ($F = 11.09$; df = 1, 119; $P = 0.001$).

Discussion

This study demonstrated that *Elaphropus xanthopus* adults can be exposed to Cry34Ab1 toxin through consumption of Cry34/35Ab1 pollen in the laboratory, and via an unknown pathway in Cry34/35Ab1 field corn. In laboratory studies, small concentrations of Cry34Ab1 protein were consistently detected from *E. xanthopus* following consumption of Cry34/35Ab1 pollen. Half-life of detectability was relatively short (11 h; Figure 3.1), suggesting that detection of pollen-borne Cry34Ab1 in field collected specimens would be unlikely, especially as several factors may accelerate antigenic decay (Harwood and Obrycki 2006), and diet breadth and consumption rate in the field are unknown for *E. xanthopus*. Despite consumption of a single pollen meal by a relatively small beetle (≈2 mm body length), positive results for Cry34Ab1 presence was consistent for *E. xanthopus* at post-consumption periods ranging from 0-36 h. Detection of Cry34Ab1 from *E. xanthopus* collected from Cry34/35Ab1 and nearby non-Bt plots was also consistent and at higher concentrations than observed in the laboratory, suggesting the rate of *E. xanthopus* exposure to Cry34Ab1 in the field may have been more frequent and/or at higher levels compared to a single pollen meal.

Study findings suggest that ground-dwelling beetles consuming Bt pollen may be exposed to a fraction of the total toxin present in the meal. This study was the first to quantify Cry protein content in frass material for a nontarget carabid. Concentrations of Cry34Ab1 in pollen-only frass material from *E. xanthopus* yielded ≈37% of the protein found in undigested Cry34/35Ab1 pollen. This study did not examine Cry34Ab1 fate

within the digestive tract, nor could the selected assay distinguish between gut-bound and unbound protein. However, a recent study suggests Cry protein is metabolized by lacewing predators feeding on intoxicated prey (Wei et al. 2008), which may be the case for the remaining ≈60% of Cry34Ab1 protein consumed by *E. xanthopus* adults. Detailed work addressing these issues may allow for a more complete estimate of potential exposure. Nonetheless, detection of toxin presence in frass material enables a more accurate calculation of exposure (dose) at the organism level, and a more precise definition of exposure for laboratory-based assays for nontarget organisms.

Transgenic toxin exposure for large-bodied carnivorous carabids has rarely been detected (Zwahlen and Andow 2005, Álvarez-Alfageme et al. 2009, Peterson et al. 2009), but the frequency of exposure in the present study was comparatively high for the small-bodied *E. xanthopus* (57%). Some carnivorous carabids may be less likely exposed if they are more apt to receive Cry proteins via diluted pathways (e.g. intoxicated prey) rather than directly from transgenic plants (Harwood et al. 2006, Álvarez-Alfageme et al. 2009). Toxin dilution was suggested as an explanation for reduced susceptibility at the tissue level for target taxa fed *B. thuringiensis* spores and inert food particles (Ben-Dov et al. 2003). Where primarily carnivorous species are desirable for biomonitoring, small-bodied carabids in the tribe Bembidiini (e.g. *Bembidion*, *Elaphropus*) may receive direct exposure to Bt proteins at high doses relative to body size, especially where omnivorous tendencies are exhibited during exposure events including anthesis. Positive detection for a high percentage of *E. xanthopus* adults relative to previous work on larger carabids suggests that small beetles may be more exposed at the population level and are perhaps a

more consistent bioindicator of exposure. Additionally, small-bodied species may enable faster sample preparation for ELISA screening, as gut dissections are not necessarily required (Peterson et al. 2009), allowing study designs to incorporate increased replication or geographic coverage in monitoring programs.

In the present study, a large proportion of *E. xanthopus* adults collected from both Cry34/35Ab1 and non-Bt field plots tested positive for Cry34Ab1 presence. While corn pollen dispersal may have contributed to cross-plot contamination, proximity of field plots (≈40 m buffer) and beetle dispersal are hypothesized to account for these results. Independent of the contamination mechanism, these results underscore the need for larger test-plot size in community studies examining transgenic toxins. Experimental design for nontarget field studies monitoring ground beetle populations will need to compensate for variability introduced by ground beetle species that may travel considerable distances (den Boer 1970, Baars 1979).

Beetles collected from the Cry34/35Ab1 field plot yielded a Cry34Ab1 concentration of 11 ng mg^{-1}. Based on the zero-hour beetles from the laboratory half-life study (6 ng mg^{-1}), *E. xanthopus* field exposure translated to approximately two Cry34/35Ab1 pollen meals. Given that 37% of Cry34Ab1 is expected to be expelled as frass (assuming pollen-only diet), actual exposure may be significantly lower. The sensitivity of ELISA allows for detection of very small antigen concentrations (e.g. ≥1 ng mg^{-1}), though the method does not distinguish between intact, active proteins and degraded, inactive protein fragments that could elicit false positive detections. While this study and previous work

(Harwood et al. 2005, Peterson et al. 2009) have established that carabid predators are being exposed to Bt proteins, it is not clear if those proteins were in a biologically active (toxic) form. For microbial formulations of whole organism Bt, toxin inactivation has been attributed to deteriorating effects caused by factors including ultra-violet light (Bauer 1995). To fully characterize exposure to transgenic toxins including Cry proteins, future work may couple exposure estimation with bioassay using susceptible species and the exposure form of the toxin to determine if toxicity is possible. Therefore, caution is warranted when interpreting values from field collected specimens as they may overestimate organism exposure.

While the pathway of Cry34Ab1 exposure is unknown for field collected *E. xanthopus*, choice and no-choice food studies (Chapter 2) suggest pollen is likely consumed in the field, whereas other potential sources of Cry34Ab1 generally are not (e.g. anthers, collembola except for scavenging, soil mite adults). However, corn pollen consumption by *E. xanthopus* in the field has not been documented, and alternative prey (fly eggs) are preferred over corn pollen in the laboratory (Chapter 2). In addition to pollen feeding, uptake of Cry34Ab1 by *E. xanthopus* may also have occurred through imbibing of Cry34Ab1-containing liquids from the substratum (direct exposure), or consumption of foods contaminated with Cry34Ab1.

This study estimated organism-level exposure for an abundant ground-dwelling predator using a realistic, direct exposure pathway. Given the relatively short period of anthesis (≈14 d), and that additional studies found no effects of Cry34/35Ab1 pollen consumption

on longevity or behavior (foraging, frass deposition) for *E. xanthopus* (Chapter 4), we conclude that field exposure to Cry34/35Ab1 pollen is unlikely to pose hazard to *E. xanthopus* adult performance. In order to produce a life-cycle description of potential risk for *E. xanthopus*, future studies may include fitness estimates under Cry34/35Ab1 exposure through a direct route. Finally, future characterization of nontarget exposure to proteinaceous gut-toxins should consider protein in frass material, which may enable more accurate estimates of exposure.

Estimation of nontarget effects at the organism level begins with Tier I-type laboratory tests that incorporate dietary exposure to the purified proteins that possess insecticidal properties (US EPA 1996, Duan et al. 2006,). Extrapolating Tier I results to field situations is dependant upon knowledge of in-field protein exposure. The present work defined laboratory dosing of a beetle-active transgenic toxin by way of pollen feeding, provided reference values for interpretation of Cry protein exposure levels acquired from field collected specimens, and revealed Cry protein exposure for a significant proportion of the *E. xanthopus* population sampled from a field corn system. Field based estimates and data from half-life of detectability and frass studies cumulatively enable estimation of organism-level exposure, and are utilized in subsequent toxicity studies examining Cry34/35Ab1 pollen feeding by *E. xanthopus* (Chapter 4).

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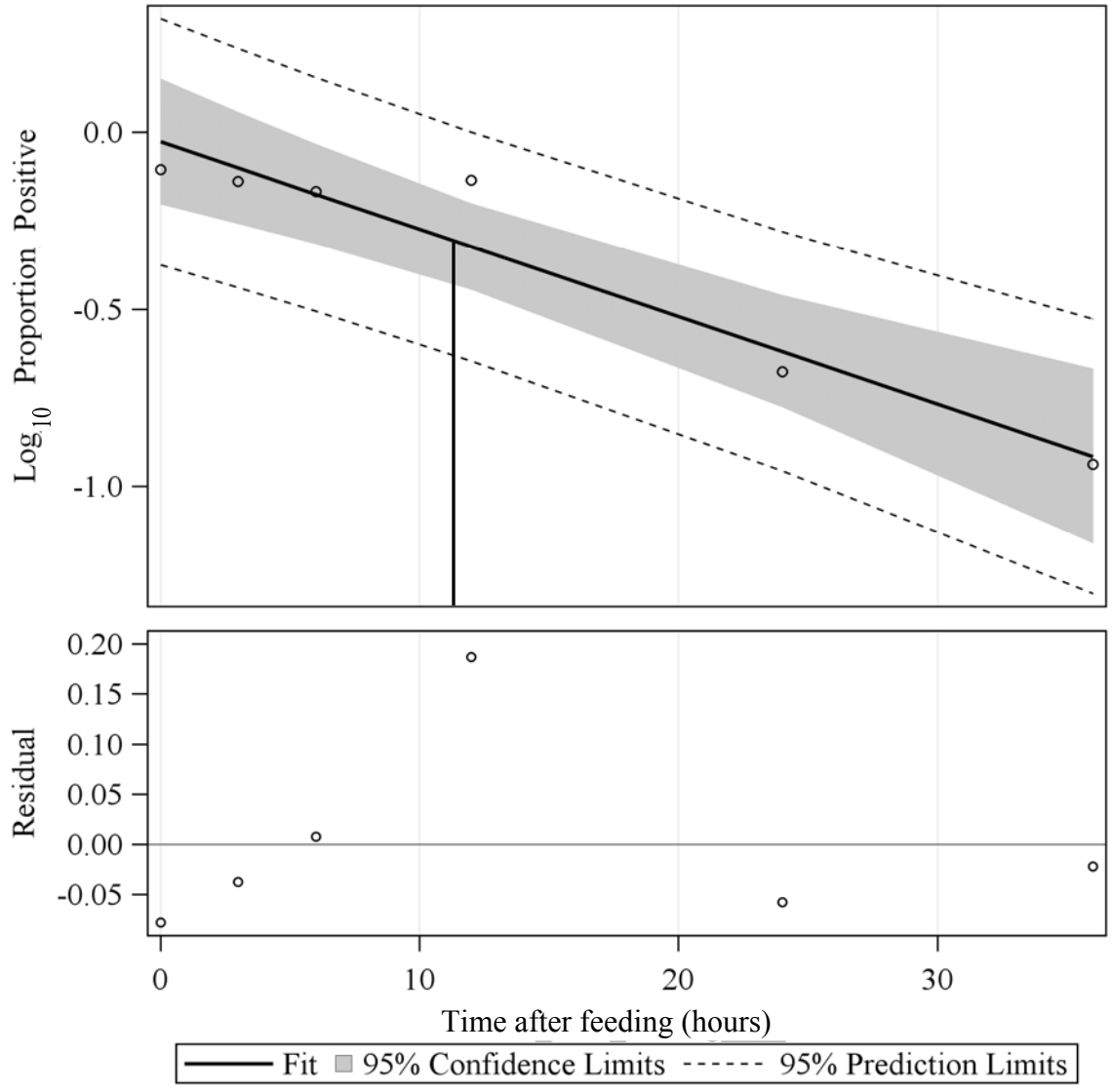

Figure 3.1. Half-life of detectability for Cry34Ab1 following consumption of Cry34/35Ab1 corn pollen by *Elaphropus xanthopus* adults. The regression equation is the Log_{10} proportion of beetles testing positive for Cry34Ab1 (y) = -0.027 -0.025*time (R^2 = 0.927, n = 6, P = 0.002). Detectability half-life (solid vertical line) is calculated by solving for time when y = - 0.3010 (i.e. Log_{10} of 0.5) (*after* Greenstone and Hunt 1993). For post-consumption periods greater than 36 h, no beetles tested positive for Cry34Ab1 presence.

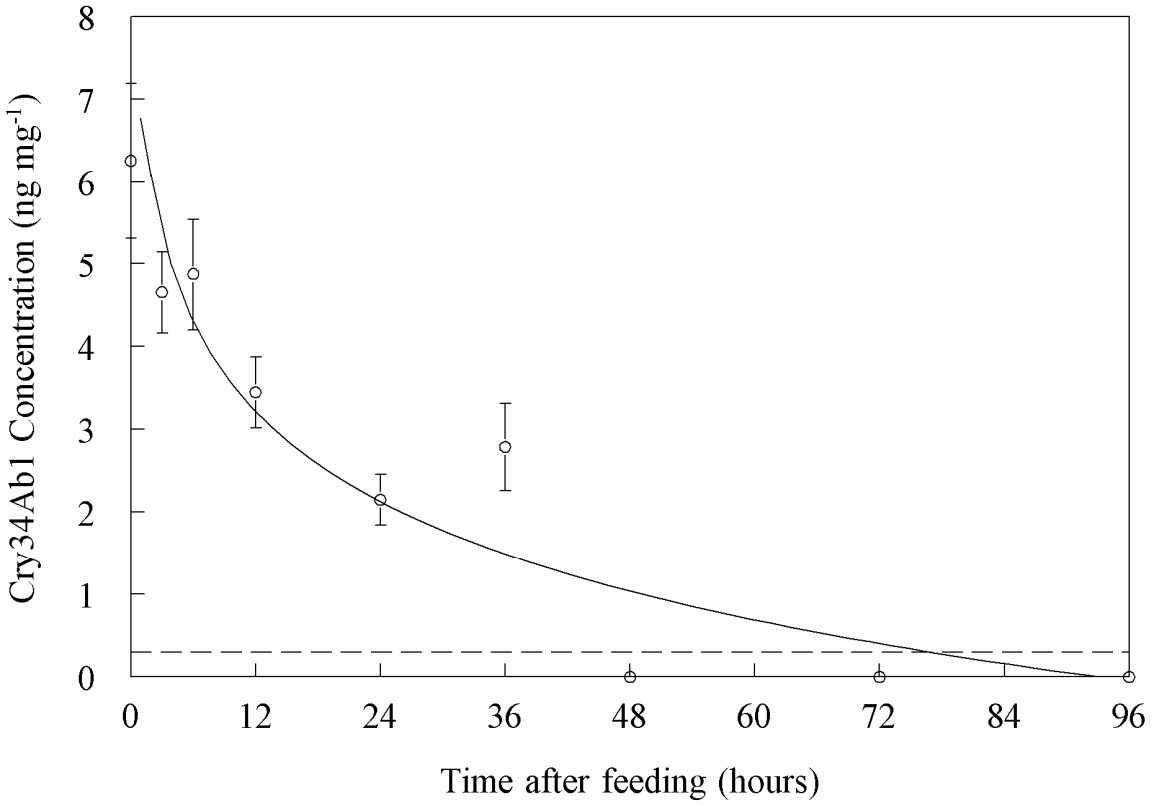

Figure 3.2. Detection and concentration of Cry34Ab1 following consumption of Cry34/35Ab1 corn pollen by *Elaphropus xanthopus* adults. Decay rate is determined by regression of concentration versus time; mean Cry34Ab1 concentration = 6.708 - 1.371*ln(time) ($R^2 = 0.844$, n = 7, $P = 0.004$). Hatched line represents the threshold for detection (0.3 ng mg^{-1}), values below which are considered to contain no significant quantities of Cry34Ab1.

CHAPTER 4: Performance effects following exposure to Cry34/35Ab1 and pyrethroid-laced corn pollen by a ground beetle, *Elaphropus xanthopus* (Coleoptera: Carabidae), and rove beetle, *Strigota ambigua* (Coleoptera: Staphylinidae)

Abstract

A laboratory study examined the effects of exposure to rootworm-resistant Bt corn pollen (Cry34/35Ab1) by adults of a ground beetle, *Elaphropus xanthopus* (Dejean) (Coleoptera: Carabidae), and rove beetle, *Strigota ambigua* (Erichson) (Coleoptera: Staphylinidae). Field collected adults were fed pollen from either Cry34/35Ab1 field corn or a non-Bt isoline. Duplicate pollen treatments containing a dilute broad-spectrum insecticide (λ-cyhalothrin) were included as positive controls. Longevity and sub-lethal behavioral responses (grooming, searching, foraging, frass deposition) were examined. No negative effects of exposure to Cry34/35Ab1 pollen were detected for either species. Conversely, detrimental effects of pyrethroid exposure included decreased longevity and increased grooming for *S. ambigua*, and decreased foraging and frass deposition for *E. xanthopus*. These findings suggest that field exposure to Cry34/35Ab1 proteins by way of pollen would not result in toxicity for either *E. xanthopus* or *S. ambigua*. Results also indicate pollen feeding by both species may increase longevity in the absence of alternative food sources.

Key Words: *Bacillus thuringiensis*, nontarget organisms, risk assessment, toxicity assay

Introduction

Risk assessment for pesticides expressed by genetically transformed plants has become an important priority for the global regulatory community. A recently developed transgenic field corn event expresses binary Cry34Ab1 and Cry35Ab1 (Cry34/35Ab1) proteinaceous toxins, conferring resistance to plants against root-feeding beetle pests in the genus, *Diabrotica* (Coleoptera: Chrysomelidae) (US EPA 2005). Transgenic Cry proteins are derived from gene isolates of the common soil bacteria, *Bacillus thuringiensis* (Bt). Cry34/35Ab1 comprise two of the four transgenic toxins (Cry3Bb1, Cry34/35Ab1, mCry3A) currently registered in the US for control of beetle pests. While Cry34/35Ab1 corn and related rootworm-resistant Cry proteins specifically target *Diabrotica* spp., unintended effects may arise through exposure to nontarget organisms (e.g. beneficial or benign species) and the subsequent potential for lethal or sub-lethal effects. Cry34/35Ab1 corn expresses Cry proteins throughout plant tissues (e.g. root, stalk, leaves, pollen, grain) (US EPA 2005). Whole plant expression of rootworm-resistant Cry proteins establishes conditions for potential exposure and injury to nontarget beetle species.

Nontarget beetles include a large assemblage of ground-dwelling species in the families Carabidae and Staphylinidae. These beetle families are well represented in arable environments, and are recognized for their contribution to pest population regulation (biological control) in managed habitats (Poehling et al. 1985, Dennis et al. 1990, Dennis and Wratten 1991, Clark et al. 1994, Dennis and Sotherton 1994, Kromp 1999, Chen et

al. 2000). Carabid and staphylinid beetles are considered useful indicators of ecological change (Eyre et al. 1989, Luff 1996, Bohac 1999, Boscaini et al. 2000) because they are sensitive to habitat disturbance (Dritschilo and Erwin 1982) including changes induced by conventional pesticides (Epstein et al. 2000, Kunkle et al. 2001).

Ground-dwelling beetles inhabiting transgenic field corn systems have been shown to contain Cry proteins (Zwahlen and Andow 2005, Harwood et al. 2005, Álvarez-Alfageme et al. 2009, Peterson et al. 2009, Chapter 3). Among potential exposure pathways (Peterson et al. 2009), uptake of intact Cry proteins is most likely through direct consumption of fresh plant tissue or exudates. Many ground-dwelling beetles exhibit omnivory, which may increase the potential for direct consumption of Bt corn tissues. For example, corn anthesis may be viewed as an exposure event (Obrist et al. 2006, Álvarez-Alfageme et al. 2009, Chapter 3); during which approximately $2x10^6$ to $5x10^6$ pollen grains are released per plant (Hoeft et al. 2000), resulting in densities in excess of $1x10^3$ grains cm^{-2} at ground level (M.D. Lepping, unpubl. data). At the organism-level, ground beetles have been exposed to Cry proteins through pollen feeding in the laboratory (Chapter 3), suggesting corn pollen is a realistic delivery vehicle for transgenic toxins. Although there is little evidence that ground-dwelling carabids actively consume pollen resources in the field (Lundgren 2009), incidental pollen uptake may occur for small-bodied species (Mitchell 1963, Dawson 1965). Additionally, corn pollen is readily consumed in captivity (Chapter 2) where it may sustain carabid adults for extended periods (Mullin et al. 2005).

To date, no studies have documented direct negative effects of Bt crops on carabid performance or fitness. In a non-controlled study, Mullin et al. (2005) found no effect of long term Cry1Ab/c and/or Cry3Bb1 pollen feeding on adult survival for 16 carabid species in 10 genera (*Agonum*, *Amara*, *Anisodactylus*, *Bembidion*, *Chlaenius*, *Harpalus*, *Patrobus*, *Poecilus*, *Pterostichus*, and *Scarites*). Ahmad et al. (2006) found no effect of Cry3Bb1 pollen-treated dog food on survival for *Harpalus caliginosus* or *H. pensylvanicus* adults, including no effects on fecundity or egg viability for *H. caliginosus*. Duan et al. (2006) found no effects on development or survival for *Poecilus chalcites* larvae reared on Cry3Bb1-laced diet. Ferry and colleagues found no effects on fitness, weight gain, or survival for *Pterostichus madidus* adults consuming Cry1Ac-fed *Plutella xylostella*, and no effects on behavior, body mass, fitness, prey consumption, or survival for *Nebria brevicollis* adults consuming Cry3A-fed *Lacanobia oleracea* (Ferry et al. 2006, 2007 respectively). Harwood et al. (2006) found no exposure or effect on fecundity for *Scarites subterraneus* adults consuming Cry1Ab-fed *Deroceras laeve*. Finally, Álvarez-Alfageme et al. (2009) found no developmental or mortality effects for *Poecilus cupreus* reared on Cry1Ab-fed *Spodoptera littoralis*. However, potential direct negative effects have been reported for non-transgenic, microbial *B. thuringiensis* formulations, where 10-15% mortality was observed for *Bembidion lampros* adults during soil exposure, although tolerance was concluded (Obadofin and Finlayson 1977). While no studies have found direct effects of Cry protein-fed prey on carabid activity, there are observations of indirect prey-mediated effects (e.g. via reduced prey quality), including reduced consumption (Cry3A; Riddick and Barbosa 2000), higher larval mortality (Cry1Ab; Meissle et al. 2005) and trends for lower adult survival (Cry1Ac;

Ferry et al. 2006). Few laboratory studies have examined effects of transgenic proteins on staphylinid beetles, although guidelines have been advanced for parasitic species such as *Aleochara bilineata* (Grimm et al. 2000). While No Observed Effect Concentrations (NOEC) have been estimated for nontargets including coccinellids (Poletika and Storer 2006), the spectrum of Cry34/35Ab1 activity has not been characterized for carabid or staphylinid species via direct exposure.

Dively et al. (in prep.) examined carabid abundance in a community study comparing Cry34/35Ab1 field corn to a non-Bt isoline variety. Their study found no differences in carabid species diversity or abundance between Bt and non-Bt treatments. The finding of no differences is generally predicted at the population level where subtle toxicity may not be manifested to a degree that would enable detection (Prasifka et al. 2008).

To assess hazard (toxicity) as part of a risk assessment program for Cry34/35Ab1 field corn, the current study examined the effects of Cry34/35Ab1 corn pollen consumption on carabid and staphylinid beetle performance (longevity and behavior) in the laboratory. Additional treatments included pollen from a non-Bt near isoline (negative control), and pollen dosed with a conventional, neurotoxic pesticide (positive control). Ground beetle exposure to neurotoxic pesticides would be expected to result in increased mortality, as well as sub-lethal effects, including: increased grooming behavior, locomotory difficulty, and paralysis (Kunkle et al. 2001). Similar effects might be expected from exposure to Bt toxins, where negative outcomes at the organism-level could include increased mortality due to reduced foraging behaviors.

Study objectives included: (1) testing for acute and chronic direct effects following Cry34/35Ab1 pollen consumption; (2) comparison of Cry34/35Ab1 dietary exposure to effects of a known toxin (λ-cyhalothrin); and (3) examination of pollen availability on beetle longevity.

Materials and Methods

Study organisms

Study organisms included a ground beetle, *Elaphropus xanthopus* (Dejean) (Coleoptera: Carabidae: Trechitae: Bembidiini), and a rove beetle, *Strigota ambigua* (Erichson) (Coleoptera: Staphylinidae: Aleocharinae: Athetini). *Elaphropus xanthopus* (Dejean 1831) is an abundant, small-bodied (≈2 mm length) micro-invertebrate predator and scavenger (Chapter 2), distributed throughout eastern North America (Bousquet and Larochelle 1993) and inhabits Maryland corn growing regions (Chapter 1). A previous study found exposure to Cry34Ab1 for *E. xanthopus* during anthesis in field corn (Chapter 3). *Strigota ambigua* (formerly *Homolota/Atheta ambigua*) (Erichson 1839, Gusarov 2003) is a small-bodied (≈2 mm length) species reported from 14 U.S. states including Maryland (Gusarov 2003, Leslie et al. 2007). In an agricultural research system, *S. ambigua* was identified as an abundant, sub-dominate species generally associated with non-Bt crop plots in contrast to plots planted with transgenic varieties including beetle-active Cry3Aa potato (Leslie et al. 2007).

Beetle collection

Beetles used in studies were collected in field corn at the U.S. Department of Agriculture (USDA), Beltsville, MD. Collection was conducted during anthesis in 0.11 ha plots

established with either Cry34/35Ab1 field corn or its non-Bt near isoline variety (Dow AgroSciences, Indianapolis, IN). Beetle collection was accomplished by vacuuming soil and detritus surrounding corn plants. To reduce the likelihood of pre-experiment exposure of beetles from non-Bt plots to Bt pollen, separate equipment was used to collect beetles in Cry34/35Ab1 and non-Bt plots. Vacuum samples were emptied into collection trays and adult *E. xanthopus* and *S. ambigua* were aspirated into vials containing moist filter paper. Vials containing beetles were placed in coolers and transported to the laboratory, and then held in a growth chamber at 20°C until experiment the following day.

Pollen collection and toxin expression

Corn pollen used in studies was collected from Cry34/35Ab1 and non-Bt field corn plots at the University of Maryland's Central Maryland Research and Education Center (CMREC), Beltsville, MD. Pollen was collected directly from plants by shaking tassels inside large paper bags, then transported to the laboratory, meshed to remove contaminants, and stored at -20°C. Compared to Cry35Ab1, Cry34Ab1 concentrations are consistently detectable and predict toxicity in susceptible targets (Herman et al. 2002). Using quantitative enzyme-linked immunosorbent assay (ELISA) (Chapter 3), Cry34/35Ab1 pollen utilized in the present study was determined to contain ≈34.2 ± 1.1 $\mu g\ g^{-1}$ Cry34Ab1 (cf. US EPA 2005). Cry35Ab1 concentrations were not estimated.

Bioassay

Bioassay was conducted in the laboratory and incorporated recommendations for testing transgenic proteins on carabid and staphylinid adults outlined by Heimbach et al. (2000) and Grimm et al. (2000), respectively. Experiments for each species were arranged as a randomized complete block design, where each block served as a replicate. Treatment structure was a 3x2x2 incomplete factorial, with three levels of pollen availability (pollen absent, limited pollen, and unlimited pollen), two levels of conventional pesticide (present or absent), and two levels of transgenic pesticide (present or absent). Treatment combinations of pollen absent or pollen limited with the conventional pesticide were not included to reduce the potential for dermal exposure. Consequently, the treatment structure was an incomplete factorial, where the resulting treatment combinations were: (1) no food; (2) limited non-Bt pollen; (3) limited Cry34/35Ab1 pollen; (4) unlimited non-Bt pollen; (5) unlimited Cry34/35Ab1 pollen; (6) unlimited non-Bt pollen with conventional pesticide; and (7) unlimited Cry34/35Ab1 pollen with conventional pesticide. The 'limited' and 'unlimited' pollen treatments were based on stock suspensions of 0.040 mg mL^{-1} dH_2O and 0.40 mg mL^{-1} dH_2O, respectively. Treatments were constructed by pipetting a 5 μL aliquot of pollen solution onto Petri dish filter paper to form individual pollen cakes. The number of pollen grains in a single pollen cake was estimated using a method outlined by Kearns and Inouye (1993), where five samples were counted twice by different researchers. The number of pollen grains in representative doses were similar and contained: 392 ± 18 grains (0.040 mg mL^{-1} non-Bt); 422 ± 10 grains (0.040 mg mL^{-1} Cry34/35Ab1); 3167 ± 89 grains (0.40 mg mL^{-1} non-Bt); and 3153 ± 111 grains (0.40 mg mL^{-1} Cry34/35Ab1). Correspondingly, pollen

availability was estimated at ≈400 grains per 2 d for the 'limited' treatment, and ≈3000 grains per 2 d for the 'unlimited' treatment. If a treatment included pyrethroid insecticide (λ-cyhalothrin; Warrior®, Syngenta Crop Protection, Greensboro, NC), a 5 µL aliquot of diluted concentrate was pipetted onto the pollen cake, and allowed to dry. Concentrated pyrethroid insecticide was serially diluted ($2x10^4$ dil) to yield ≈27 ng active ingredient per 5 µL aliquot. Pyrethroid dosing was chosen to be similar to the oral LD_{50} value for honeybee larvae (38 ng bee^{-1}) receiving a single dose of λ-cyhalothrin (USDA 2004). λ-cyhalothrin has been found to disturb carabid populations in cropping environments (Devotto et al. 2007, Rose and Dively 2007), and is classified as a broad-spectrum pesticide.

After treatments were prepared, a single beetle was sealed in a Petri dish containing one of the seven treatments, and dH_2O was added to Petri dishes in order to maintain moisture. Finally, all Petri dishes within a single replicate were maintained in resealable plastic bags kept at ambient temperature on laboratory bench shelves. Treatments were reapplied every 2 d to prevent water condensation and the growth of mold. Beetles that died due to non-treatment factors were removed from the data set. Studies included 39 replicates of each treatment for *E. xanthopus*, and 15 replicates of each treatment for *S. ambigua*. As pre-experimental exposure to Cry-proteins may have influenced beetle longevity, the effect of field plot treatment (origination effects) was examined in preliminary analyses, where no effect was detected and variation due to this factor blocked in the final analyses.

Data Collection and Analysis

Longevity

Longevity (number of days alive) was calculated for each beetle, and natural log transformed to achieve normality prior to analysis. The effect of pollen availability on longevity was examined using ANOVA (PROC MIXED; SAS Institute Inc. 2008), and Tukey-Kramer tests were used to limit experimentwise error rates ($\alpha = 0.05$). To examine the effect of pesticide on longevity (2x2 factorial), contrast tests for presence versus absence of conventional and transgenic pesticides were performed.

Behavior

Behavioral responses were monitored to examine sub-lethal (chronic) treatment effects. Behavioral observations were recorded every 2 d (1 d after pollen treatment application), and scored as binary data (absence or presence). Behaviors included: grooming (e.g. cleaning antennae and/or tarsi, scratching abdomen with leg); searching (e.g. walking movement with antennae and/or palpi contact on substratum [random searching]); foraging (e.g. evidence of previous feeding via disruption of the pollen cake [directed searching]); and frass deposition. The frequency of each behavior was calculated as the number of times a behavior was observed (instances) divided by the total number of observations and multiplied by 100 to produce a percentage.

Behavioral data used in analysis were from treatment combinations of the primary 2x2 factorial (presence/absence of conventional/transgenic pesticides) at the level of

unlimited pollen availability. Data were analyzed for time periods when mortality across treatments was <50% (0-51 d for *E. xanthopus*; 0-21 d for *S. ambigua*). Behavioral responses were analyzed as repeated measures, and compared using contrast tests in the GLIMMIX procedure (SAS Institute Inc. 2008).

Results

Longevity

Corn pollen as a singular food source sustained both beetle species for extended periods. Longevity increased with increasing corn pollen availability for both *E. xanthopus* (F = 105.29; df = 2, 120; $P < 0.001$) and *S. ambigua* (F = 107.88; df = 2, 28; $P < 0.0001$) (Figure 4.1). For *E. xanthopus*, contrast tests for the 2x2 factorial (transgenic and conventional pesticides) did not detect differences for the conventional (F = 0.47; df = 1, 154; P = 0.494) or transgenic pesticide (F = 1.52; df = 1, 154; P = 0.220) (Figure 4.2). For *S. ambigua*, contrast tests for the same 2x2 factorial showed reduced longevity due to the conventional insecticide (F = 8.36; df = 1, 40.6; P = 0.006), but no effect of the transgenic pesticide (F = 0.54; df = 1, 40.6; P = 0.465) (Figure 4.3).

Grooming

Grooming behavior in *E. xanthopus* was infrequent (2.5%; 71 instances out of 2800 observations) and did not allow for statistical analysis. Grooming behavior in *S. ambigua* was infrequent (15.1%; 61 instances out of 403 observations), and was increased by the conventional insecticide (F = 6.59; df = 1, 29.72; P = 0.0156), but not the transgenic pesticide (F = 2.26; df = 1, 29.72; P = 0.1432) (Table 4.1).

Searching

Elaphropus xanthopus searching was infrequent (9.2%; 258 instances out of 2800 observations) and was not influenced by the conventional ($F < 0.01$; df = 1, 179.3; $P = 0.9815$) or transgenic pesticide ($F = 0.15$; df = 1, 179.3; $P = 0.6951$). *Strigota ambigua* searching was frequent (54.7%; 211 instances out of 386 observations) but was not influenced by the conventional ($F = 0.47$; df = 1, 80.47; $P = 0.4963$) or transgenic pesticide ($F = 0.32$; df = 1, 80.47; $P = 0.5758$) (Table 4.1).

Foraging

Foraging at the pollen cake by *E. xanthopus* was frequent (70.8%; 1955 instances out of 2762 observations), and was significantly reduced by the conventional pesticide ($F = 29.91$; df = 1, 195.5; $P < 0.0001$) but not the transgenic pesticide ($F = 2.74$; df = 1, 195.5; $P = 0.0994$). Foraging at the pollen cake by *S. ambigua* was frequent (60.0%; 173 instances out of 290 observations), but was not influenced by the conventional ($F = 1.95$; df = 1, 31.35; $P = 0.1728$) or the transgenic pesticide ($F = 3.46$; df = 1, 31.35; $P = 0.0723$) (Table 4.1).

Frass deposition

Frass deposition by *E. xanthopus* was frequent (76.2%; 474 instances out of 622 observations), and was significantly reduced by the conventional ($F = 8.37$; df = 1, 158.4; $P = 0.0043$) but not the transgenic pesticide ($F = 2.25$; df = 1, 158.4; $P = 0.1355$). Frass deposition by *S. ambigua* was infrequent (30.9%; 42 instances out of 136 observations),

and was not influenced by the conventional ($F = 2.27$; df = 1, 25.83; $P = 0.1436$) or the transgenic pesticide ($F = 0.11$; df = 1, 25.83; $P = 0.7459$) (Table 4.1).

Discussion

Future government registration requirements for beetle-active transgenic toxins may include risk assessment for nontarget species in the families Carabidae and/or Staphylinidae (Rose 2007). The primary objective of this study was to examine the effects of rootworm-resistant corn pollen on two nontarget ground-dwelling beetles: a ground beetle, *Elaphropus xanthopus* (Dejean); and a rove beetle, *Strigota ambigua* (Erichson). This study demonstrated there were no detectable effects of Cry34/35Ab1 pollen consumption on longevity (Figures 4.2 and 4.3) or behavior (Table 4.1) for either beetle species compared to beetles fed non-Bt pollen treatments. Availability of corn pollen in the field is relatively limited compared to treatments in the present study. The finding of no negative effects following continuous and artificially high Cry34/35Ab1 pollen exposure in the laboratory suggests that consumption of Cry34/35Ab1 pollen in the field during anthesis is not likely pose a hazard to *E. xanthopus* or *S. ambigua* adult performance. These conclusions agree with those of similar studies that have not detected performance or fitness effects for other carabid species following exposure to rootworm-resistant and/or lepidopteran-resistant transgenic proteins (Mullin et al. 2005, Harwood et al. 2006, Álvarez-Alfageme et al. 2009).

For foliar-dwelling coccinellid predators that may consume pollen directly from transgenic plants in the field, no significant direct effects have been reported following exposure to lepidopteran-resistant (Pilcher et al. 1997, Bai et al. 2005) or rootworm-

resistant varieties (Duan et al. 2002, Lundgren and Wiedenmann 2002, Ahmad et al. 2006), although data is periodically disputed (Rauschen 2009, Schmidt et al. 2009). A commonly assessed nontarget species, *Coleomegilla maculata* (Coleoptera: Coccinellidae), relies on pollen as a dietary component in the field (Cottrell and Yeargan 1988, Lundgren et al. 2004) and is capable of development solely on pollen (Pilcher and Obrycki 1994, Lundgren and Wiedenmann 2004). *Coleomegilla maculata* is also capable of completing development on Bt fed prey (Cry3A; Riddick and Barbosa 1998) or Bt pollen (Cry3Bb; Lundgren and Wiedenmann 2002) without any adverse effects. While no effects have been reported for carabid larvae reared on Cry protein-containing food (Cry3Bb1: Duan et al. 2006, Cry1Ab: Álvarez-Alfageme et al. 2009), few studies have examined the larval stage – the stage most susceptible to intoxication for the target group (US EPA 2005, Nowatzki et al. 2006). As immature stages of nontarget taxa are expected to be more susceptible to novel toxins than the adult stage, further development of test systems that include rearing protocols for larval stages of ground-dwelling beetle species is needed to fully examine the potential for adverse effects.

Nontarget exposure to fresh Cry34/35Ab1 corn pollen in the cropping environment is a realistic route for delivery of Cry34Ab1, but not necessarily Cry35Ab1 (US EPA 2005). Cry34/35Ab1 pollen contains marginally detectable amounts of Cry35Ab1 that are well below levels in root tissues that target species consume. Synergism of Cry34Ab1 and Cry35Ab1 results in optimal toxicity for target species but is not required, as Cry34Ab1 concentrations largely predict toxicity (Herman et al. 2002). Cry35Ab1 levels in pollen may be too low to initiate Cry34/35Ab1 synergism in susceptible species and could

further reduce the potential for nontarget toxicity. Given the transgenic toxin dose in the present study was dependent on plant expression, dosing approximated the Expected Environmental Concentration (1x EEC) rather than levels typically used in Tier I risk assessment studies (10x EEC). The concentration of active ingredient administered in this study (≈34 μg g^{-1} Cry34Ab1) may define an initial No Observed Effect Concentration (NOEC) for use in future work.

Sub-lethal measures can reveal subtle adverse effects of toxin exposure, and may provide mechanistic explanations for reductions in taxa at the population level. Sensitivity to pyrethroid toxins for foraging and frass deposition by *E. xanthopus* suggest this species may be suitable for screening future broad-spectrum toxins. Unlike *E. xanthopus*, strong negative effects were detected for *S. ambigua* under dilute pyrethroid exposure, and grooming behavior increased. Excessive grooming interferes with normal behavior and is an indicator of neurotoxin exposure (Kunkle et al. 2001). The differential pyrethroid sensitivity across species in this study is consistent with community-level patterns described by Rose and Dively (2007), who found lower staphylinid abundance in corn plots sprayed with a pyrethroid insecticide, while no effects were found for carabid taxa. These results suggest *S. ambigua* may be a suitable bioindicator organism for insecticide exposure and toxicity testing. Current limitations of utilizing staphylinid species in bioassays include the need for non-destructive techniques for species determination and taxonomic revision in some groups. For beetles fed transgenic pollen, behavioral measures suggest that Cry34/35Ab1 pollen consumption in the field would not likely disrupt behavioral functions for the two species examined. In agreement with laboratory

and field studies examining conventional and transgenic toxins (Rose and Dively 2007, Mullin et al. 2005, Leslie et al. 2009), broad-spectrum neurotoxic pesticides are considered a significant morality factor for nontarget ground-dwelling beetles, and the suite of registered Bt proteins do not introduce any detectable adverse effects. These conclusions support the position that narrow-spectrum pesticides including Cry proteins are compatible with biological control tactics as they do not influence nontarget activity.

Finally, the present study enabled examination of pollen availability on adult beetle longevity, and revealed increased longevity with increasing pollen availability for both *E. xanthopus* and *S. ambigua* (Figure 4.1). Pollen feeding in the field by both species would be expected to increase longevity, particularly when prey is scarce (Limburg and Rosenheim 2001, Coll and Guershon 2002). Consistent foraging at the pollen cake combined with sustained frass deposition (Table 4.1) and increased longevity for carabid beetles fed only corn pollen (Mullin et al. 2005, Figure 4.1) suggest that corn pollen is a useful food source for application in control treatments and a vehicle for exposure to transgenic toxins.

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Table 4.1. Frequency (percent) of behaviors for ground-dwelling beetles following exposure to corn pollen treated with λ-cyhalothrin (Pyrethroid) or containing Cry34/35Ab1 proteins[a].

Species	Behavior	Frequency (%)	Pyrethroid		Cry34/35Ab1	
			Absent	Present	Absent	Present
Elaphropus xanthopus	Grooming[b]	2.5	—	—	—	—
	Searching	9.2	9 ± 1a	5 ± 1a	8 ± 1a	6 ± 1a
	Foraging	70.8	80 ± 1a	62 ± 2b	75 ± 2a	70 ± 2a
	Frass deposition	76.2	87 ± 2a	73 ± 3b	83 ± 3a	78 ± 3a
Strigota ambigua	Grooming	15.1	11 ± 3b	19 ± 3a	14 ± 3a	16 ± 3a
	Searching	54.7	63 ± 5a	44 ± 6a	51 ± 5a	56 ± 5a
	Foraging	60	56 ± 5a	62 ± 7a	53 ± 6a	65 ± 6a
	Frass deposition	30.9	30 ± 8a	35 ±11a	35 ± 9a	30 ± 9a

[a] Behavioral frequencies were calculated as the number of occurences divided by the total number of observations x100. Frequency values for each behavior followed by the same letter within Pyrethroid or Cry34/35Ab1 groups are not significantly different ($P > 0.05$).

[b] Infrequent occurrence of grooming behavior by *E. xanthopus* precluded statistical analysis.

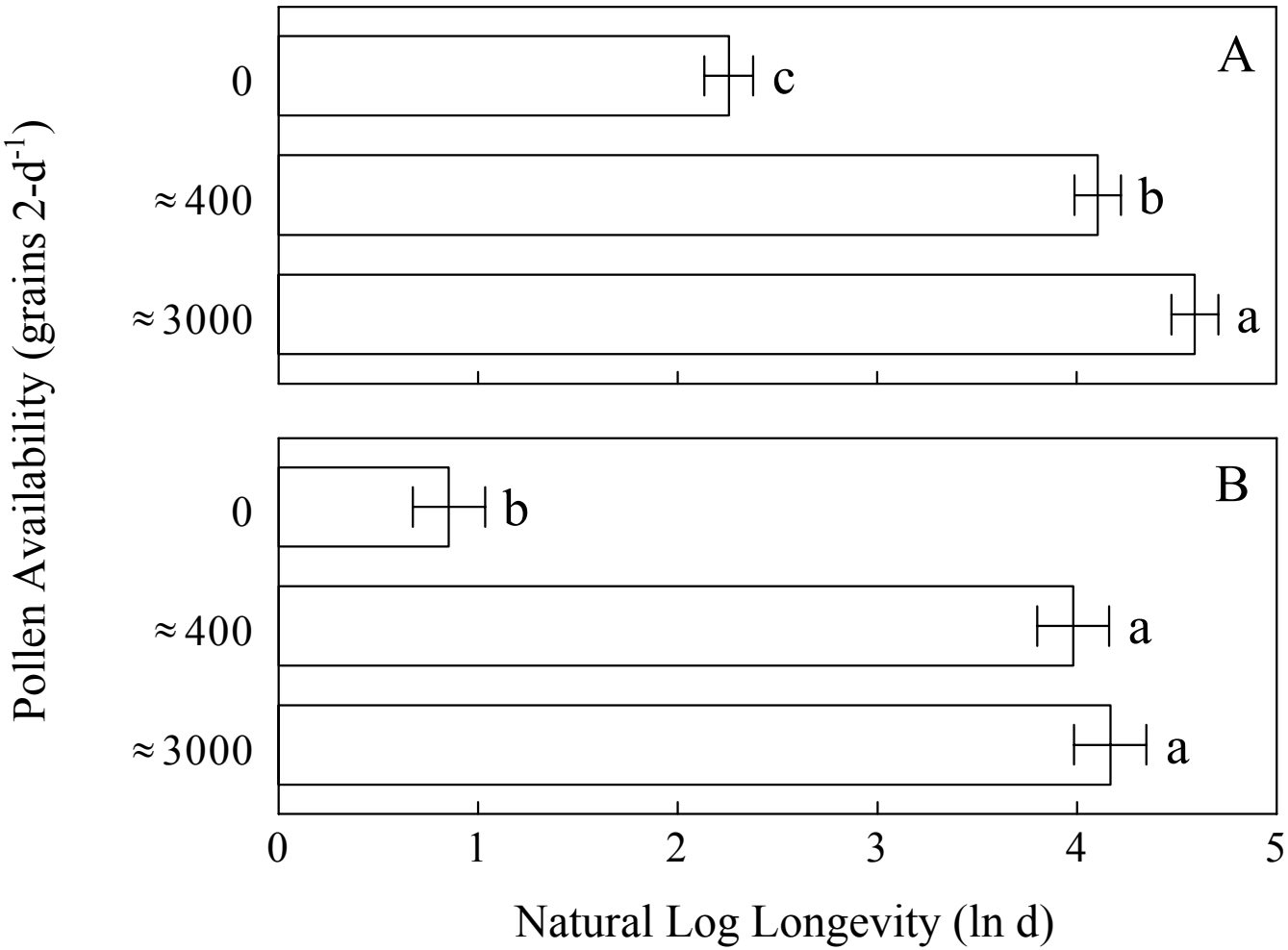

Figure 4.1. Influence of corn pollen availability (pooled across Bt and non-Bt) on longevity for adult (A) *Elaphropus xanthopus* and (B) *Strigota ambigua*.

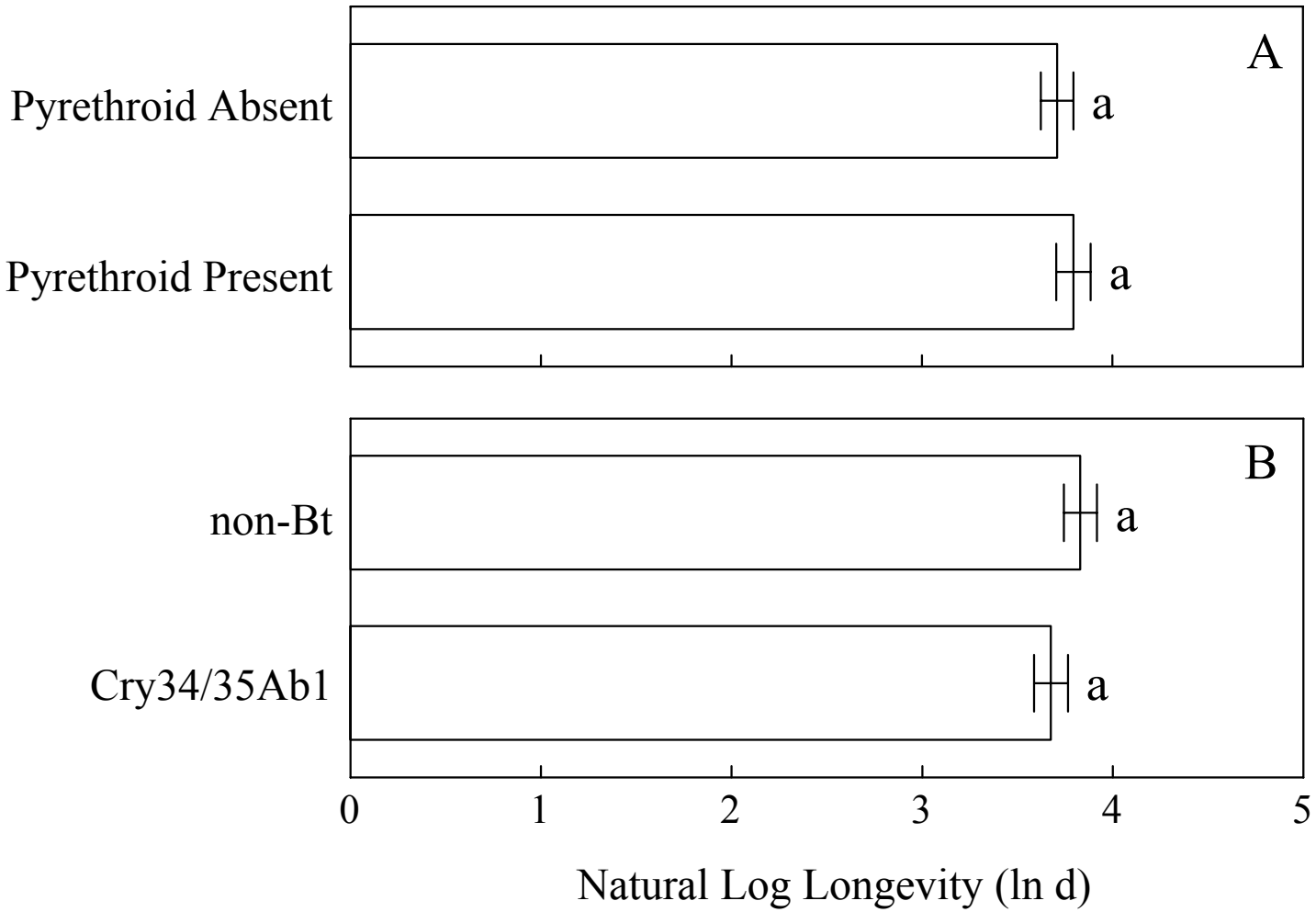

Figure 4.2. Influence of (A) λ-cyhalothrin (Pyrethroid) treated corn pollen and (B) Cry34/35Ab1 corn pollen on *Elaphropus xanthopus* adult longevity (ln transformed). Longevity values followed by the same letter within Pyrethroid or Cry34/35Ab1 are not significantly different ($P > 0.05$).

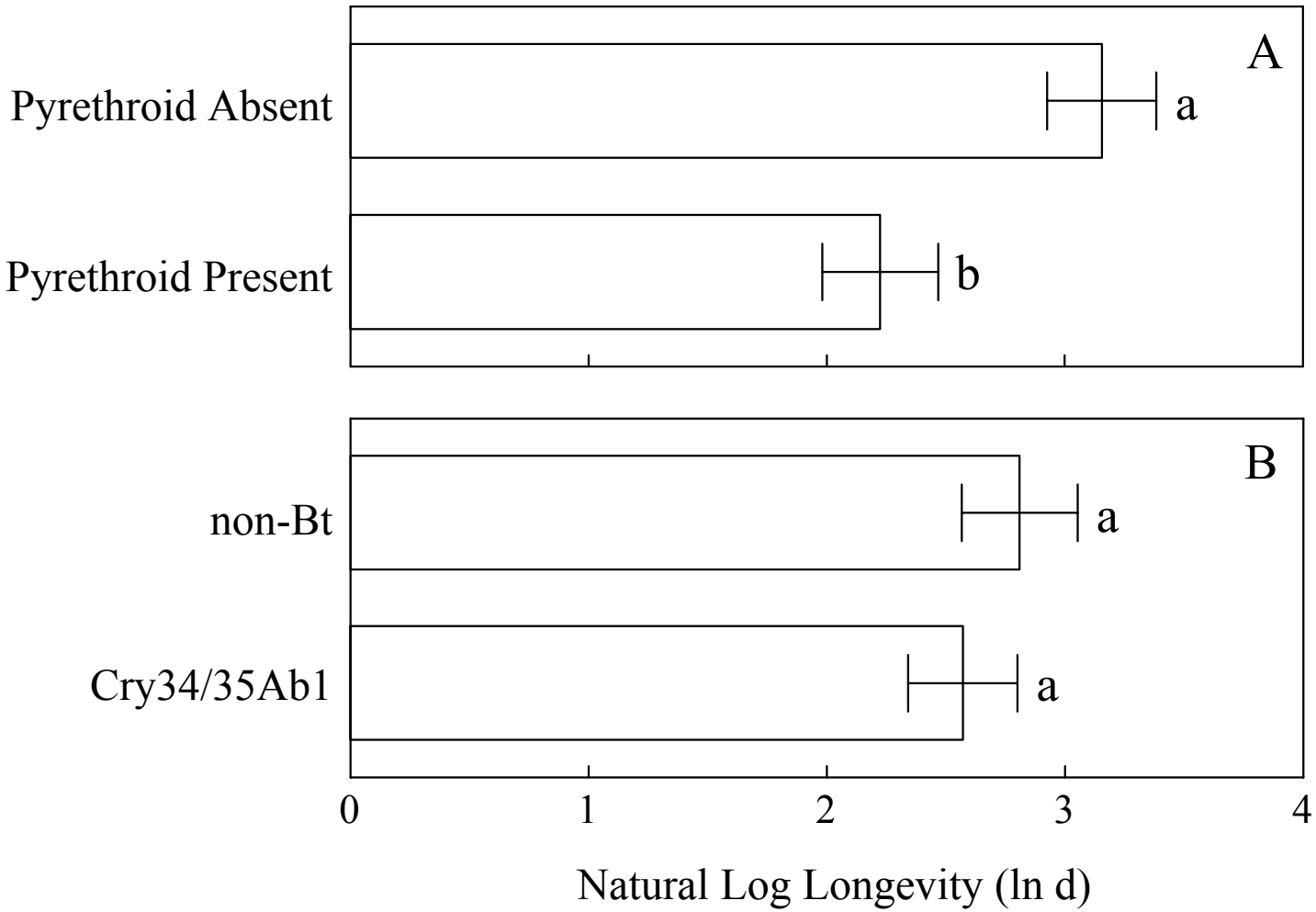

Figure 4.3. Influence of (A) λ-cyhalothrin (Pyrethroid) treated corn pollen and (B) Cry34/35Ab1 corn pollen on *Strigota ambigua* adult longevity (ln transformed). Longevity values followed by the same letter within Pyrethroid or Cry34/35Ab1 are not significantly different ($P > 0.05$).

Appendices

Appendix A. Selective factors found to influence pitfall trap capture of carabid adults, and their associated references: (A) environmental factors, (B) interaction of trap characteristics and species-specific responses.

Factor	References
A) environmental factors	
habitat type	Dufrêne and Legendre 1997
soil moisture, soil type	Luff et al. 1989, Irmler 1999, Irmler and Hoernes 2003, Magagula 2003
humidity, air or soil temperature	Briggs 1961, Greenslade 1961, Adis 1979, Honěk 1988, Nève 1994, Magura et al. 2001, Hatten et al. 2007, (visual observations: Kruse et al. 2008)
vegetation height, leaf litter cover	Greenslade 1964, Lövei and Sunderland 1996, Magura et al. 2001, Magagula 2003, Phillips and Cobb 2005, Hatten et al. 2007
daily and seasonal activity cycles	Adis 1979, Niemelä et al. 1989, Niemelä et al. 1992, Carmona and Landis 1999
diel patterns	Luff 1978, Alderweireldt and Desender 1990, Kegel 1990
hunger state	Mols 1987, Mols 1993, Frampton et al. 1995, Mauremooto et al. 1995
tillage and management practices	Clark et al. 2006, Hatten et al. 2007
crop density	Honěk 1988
lethal and sub-lethal effects of pesticides	Luff 1987
B) trap-by-species interactions	
attraction to preservatives	Luff 1975, Holopainen 1990, Lemieux and Lindgren 1999, Pekár 2002
agility, perception, running speed	Baars 1979, Desender and Maelfait 1986, Halsall and Wratten 1988, Morrill 1990, Topping and Sunderland 1992, Tonhasca 1993
trap placement	Greenslade 1964
trap shape or type	Luff 1975, Curtis 1980, Bostanian et al. 1983, Halsall and Wratten 1988, Spence and Niemelä 1994, Obrist and Duelli 1996, Buddle and Hammond 2003
trap diameter/size	Luff 1975, Work et al. 2002
body size	Lang 2000
escape via trap material	Luff 1975
trapping out (local depletion)	Digweed et al. 1995

Appendix B. Selective factors found to influence pitfall trap capture of carabid adults: References.

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Appendix B. Selective factors found to influence pitfall trap capture of carabid adults: References, Cont'd.

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Appendix C. Select studies comparing sampling methods for ground beetles (Coleoptera: Carabidae).

Methods	Citation
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pitfall trapping (open), manual collection at light tower	Liu et al. 2007
pitfall trapping (open), flight-intercept (window) trapping	Hyvärinen et al. 2006
pitfall trapping (open), manual searching	Lin et al. 2005
pitfall trapping (open), litter bagging	Prasifka et al. 2007
pitfall trapping (open), quadrat (litter washing)	Spence and Niemelä 1994
pitfall trapping (open), quadrat (soil drench)	Andersen 1995
pitfall trapping (open / fenced)	Holland and Smith 1999, Lang 2000
pitfall trapping (open / fenced), suction sampling (D-vac)	Mommertz et al. 1996
suction sampling (D-vac / G-vac)	Stewart and Wright 1995
suction sampling (Vortis), quadrat (clip-cage)	Brook et al. 2008

Appendix D. Select studies comparing sampling methods for ground beetles (Coleoptera: Carabidae): References.

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Yahiro, K. and K. Yano. 1997. Ground beetles (Coleoptera: Caraboidea) caught by a light trap during ten years. ESAKIA 37: 57-69.

Appendix E. Trophic identity assignments for carabid adults collected during study.

Taxa Code[a]	Species	Tribe	Trophic Identity[b]	Citations[c]
Acr	*Acupalpus partiarius* (Say)	Harpalini	omnivore	[Larochelle 1990], Jo and Smitley 2003, [Honek et al. 2005]
Acu	*A. pauperculus* Dej.	Harpalini	omnivore	[Larochelle 1990], [Jo and Smitley 2003], [Honek et al. 2005]
Ago	*Agonum octopunctatum* (F.)	Platynini	omnivore	[Larochelle 1990], Losey and Denno 1999, [Lundgren 2009]
Agp	*A. punctiforme* (Say)	Platynini	omnivore	Larochelle 1990, Lundgren 2009
Ame	*Amara aenea* (DeG.)	Zabrini	omnivore	Larochelle 1990, Lundgren 2009
Amg	*A. angustata* (Say)	Zabrini	omnivore	Larochelle 1990, Lundgren 2009
Amt	*A. anthobia* Vil. & Vil.	Zabrini	omnivore	[Larochelle 1990], Honek et al. 2005
Amc	*A. cupreolata* Putz.	Zabrini	omnivore	Larochelle 1990, Lundgren 2009
Amf	*A. familiaris* (Duft.)	Zabrini	omnivore	Larochelle 1990, Lundgren 2009
Ami	*A. impuncticollis* (Say)	Zabrini	omnivore	Barney and Pass 1986, Larochelle 1990
Ams	*Amphasia sericea* (Harris)	Harpalini	omnivore	Larochelle 1990, Lundgren 2009
Anc	*Anisodactylus caenus* (Say)	Harpalini	omnivore	[Larochelle 1990], Lundgren 2009
Anr	*A. rusticus* (Say)	Harpalini	omnivore	Larochelle 1990, Lundgren 2009
Ans	*A. sanctaecrucis* (F.)	Harpalini	omnivore	Larochelle 1990, Lundgren 2009
Ban	*Badister notatus* Hald.	Licinini	carnivore	[Larochelle 1990], [Arnett and Thomas 2000]
Bea	*Bembidion affine* Say	Bembidiini	carnivore	[Larochelle 1990]
Ber	*B. rapidum* (LeC.)	Bembidiini	carnivore	[Larochelle 1990]
Brr	*Bradycellus rupestris* (Say)	Harpalini	omnivore	Larochelle 1990, [Lundgren 2009]
Brt	*B. tantillus* (Dej.)	Harpalini	omnivore	[Larochelle 1990], [Lundgren 2009]
Cao	*Calathus opaculus* LeC.	Platynini	omnivore	Larochelle 1990, [Lundgren 2009]
Cas	*Calosoma scrutator* (F.)	Carabini	carnivore	Larochelle 1990
Ctr	*Chlaenius tricolor* Dej.	Callistini	carnivore	Larochelle 1990
Cip	*Cicindela punctulata* Oliv.	Cicindelini	carnivore	Larochelle 1990
Clb	*Clivina bipustulata* (F.)	Clivinini	omnivore	[Larochelle 1990], [Lundgren 2009]
Die	*Dicaelus elongatus* Bon.	Licinini	carnivore	Larochelle 1990
Dyg	*Dyschiriodes globulosus* (Say)	Clivinini	carnivore	Larochelle 1990

Appendix E. Trophic identity assignments for carabid adults collected during study, Continued.

Taxa Code[a]	Species	Tribe	Trophic Identity[b]	Citations[c]
Ela	*Elaphropus anceps* (LeC.)	Bembidiini	carnivore	Rutledge et al. 2004
Elx	*E. xanthopus* (Dej.)	Bembidiini	carnivore	Chapter 2
Haa	*Harpalus affinis* (Schr.)	Harpalini	omnivore	Lundgren 2009
Hal	*H. longicollis* LeC.	Harpalini	omnivore	Larochelle 1990, [Lundgren 2009]
Hap	*H. pensylvanicus* (DeG.)	Harpalini	omnivore	Larochelle 1990, Lundgren 2009
Has	*H. somnulentus* Dej.	Harpalini	omnivore	Larochelle 1990
Non	*Notiophilus novemstriatus* LeC.	Notiophilini	carnivore	Larochelle 1990
Pas	*Paratachys sagax* (Csy.)	Bembidiini	carnivore	[Larochelle 1990]
Pal	*Patrobus longicornis* (Say)	Patrobini	omnivore	Larochelle 1990
Poc	*Poecilus chalcites* (Say)	Pterostichini	carnivore	Larochelle 1990, O'Rourke et al. 2006
Pol	*P. lucublandus* (Say)	Pterostichini	carnivore	Best and Beegle 1977, Larochelle 1990, O'Rourke et al. 2006
Poa	*Polyderis laevis* (Say)	Bembidiini	carnivore	Larochelle 1990
Pta	*Pterostichus atratus* (Newm.)	Pterostichini	carnivore	[Larochelle 1990]
Scq	*Scarites quadriceps* Chd.	Scaritini	carnivore	[Larochelle 1990]
Scs	*S. subterraneus* F.	Scaritini	carnivore	Larochelle 1990
Seo	*Selenophorus opalinus* (LeC.)	Harpalini	omnivore	[Larochelle 1990], [Lundgren 2009]
Sep	*S. pedicularius* Dej.	Harpalini	omnivore	Larochelle 1990, [Lundgren 2009]
Stc	*Stenolophus conjunctus* (Say)	Harpalini	omnivore	Larochelle 1990, Lundgren 2009
Sto	*S. ochropezus* (Say)	Harpalini	omnivore	Larochelle 1990, [Lundgren 2009]
Str	*S. rotundatus* LeC.	Harpalini	omnivore	[Larochelle 1990], [Lundgren 2009]
Tev	*Tetracha virginica* (L.)	Cicindelini	carnivore	Larochelle 1990
Trq	*Trechus quadristriatus* (Schr.)	Trechini	omnivore	Larochelle 1990, Lundgren 2009
Trf	*Trichotichnus fulgens* (Csiki)	Harpalani	omnivore	[Loreau 1988], [Arnett and Thomas 2000]

[a] Species abbreviations (Taxa Code) correspond to taxa codes depicted in redundancy analysis biplots.

[b] Carnivore (e.g. primarily animal-based diet, including saprophagy);
Omnivore (e.g. animal and vegetal dietary components, *senso lato* granivory).

[c] Bracketed citations denote approximation of trophic identity based on records for related species.

Appendix F. Trophic identity assignments for carabid adults, Continued: References.

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Appendix G. Adult carabid beetle body metrics by species and sex.

Species	Sex	n	Median Length (mm)	Mean Length ± SE (mm)[a]	Length Range (mm)	Median Weight (mg)	Mean Weight ± SE (mg)[a]	Weight Range (mg)	Body Volume ± SE (mm^3)
Acupalpus partiarius (Say)	♀	33	3.57	3.58 ± 0.04 a	3.1 - 4.0	0.74	0.71 ± 0.03 a	0.29 - 1.09	2.4 ± 0.1
	♂	25	3.39	3.38 ± 0.03 b	3.0 - 3.7	0.61	0.57 ± 0.03 b	0.17 - 0.81	2.0 ± 0.1
A. pauperculus Dej.	♀	11	3.05	2.98 ± 0.05 ns	2.6 - 3.2	0.40	0.40 ± 0.03 ns	0.28 - 0.57	1.4 ± 0.1
	♂	14	3.03	3.05 ± 0.06 ns	2.8 - 3.5	0.36	0.40 ± 0.04 ns	0.23 - 0.65	1.4 ± 0.1
Agonum octopunctatum (F.)	♀	3	7.90	7.88 ± 0.09 ns	7.7 - 8.0	3.86	5.12 ± 1.68 ns	3.05 - 8.45	27.5 ± 2.5
	♂	4	7.58	7.61 ± 0.11 ns	7.4 - 7.9	4.19	4.63 ± 0.55 ns	3.90 - 6.24	24.0 ± 1.6
A. punctiforme (Say)	♀	11	7.82	7.93 ± 0.13 ns	7.3 - 8.5	6.43	6.64 ± 0.65 ns	3.05 - 9.92	25.9 ± 1.1
	♂	10	7.64	7.68 ± 0.06 ns	7.4 - 7.9	6.19	6.08 ± 0.52 ns	3.54 - 9.20	23.0 ± 0.8
Amara aenea (DeG.)	♀	49	7.59	7.60 ± 0.07 ns	6.8 - 10.2	7.47	7.58 ± 0.37 ns	2.00 - 11.98	28.4 ± 0.7
	♂	29	7.50	7.53 ± 0.06 ns	6.7 - 8.3	8.38	8.22 ± 0.49 ns	2.06 - 13.82	26.9 ± 0.8
A. angustata (Say)	♀	1	7.45	7.45	.	10.31	10.31	.	22.5
	♂	1	6.53	6.53	.	3.87	3.87	.	16.0
A. anthobia Vil. & Vil.	♀	1	6.41	6.41	.	4.25	4.25	.	17.4
	♂	2	6.02	6.02 ± 0.20	5.8 - 6.2	3.32	3.32 ± 1.89	1.43 - 5.21	10.7 ± 0.6
A. cupreolata Putz.	♀	1	7.00	7.00	.	5.89	5.89	.	20.9
A. familiaris (Duft.)	♀	33	6.65	6.60 ± 0.06 a	5.8 - 7.3	4.85	4.99 ± 0.31 ns	0.83 - 9.09	18.4 ± 0.5
	♂	34	6.40	6.37 ± 0.05 b	5.8 - 6.8	4.54	4.71 ± 0.26 ns	1.84 - 7.91	16.1 ± 0.4
A. impuncticollis (Say)	♀	11	8.67	8.75 ± 0.16 ns	7.8 - 9.4	13.82	12.78 ± 1.42 ns	6.39 - 18.64	40.9 ± 1.9
	♂	9	8.35	8.31 ± 0.25 ns	7.3 - 9.3	9.85	9.61 ± 1.12 ns	2.31 - 13.54	35.7 ± 3.0
Amphasia sericea (Harris)	♀	1	9.61	9.61	.	11.41	11.41	.	49.3
	♂	1	9.84	9.84	.	15.57	15.57	.	46.5
Anisodactylus caenus (Say)	♀	1	9.18	9.18	.	10.18	10.18	.	45.0
	♂	1	8.94	8.94	.	10.55	10.55	.	46.1
A. rusticus (Say)	♀	13	10.30	10.30 ± 0.15 ns	9.0 - 11.1	12.64	12.84 ± 1.96 ns	4.40 - 25.02	63.8 ± 3.1
	♂	10	10.49	10.16 ± 0.30 ns	8.1 - 11.3	17.10	17.37 ± 1.60 ns	9.88 - 25.34	60.6 ± 5.1
A. sanctaecrucis (F.)	♀	7	9.34	9.19 ± 0.21 ns	8.1 - 9.8	10.41	8.93 ± 1.34 ns	3.51 - 13.78	39.9 ± 3.6
	♂	6	9.06	9.20 ± 0.17 ns	8.9 - 10.0	10.21	9.91 ± 1.12 ns	5.50 - 12.69	42.2 ± 2.7
Badister notatus Hald.	♂	1	4.42	4.42	.	1.46	1.46	.	3.8

Appendix G. Adult carabid beetle body metrics by species and sex, Contined.

Species	Sex	n	Median Length (mm)	Mean Length ± SE (mm)[a]	Length Range (mm)	Median Weight (mg)	Mean Weight ± SE (mg)[a]	Weight Range (mg)	Body Volume ± SE (mm^3)
Bembidion affine Say	♀	1	3.09	3.09	.	0.35	0.35	.	2.0
	♂	1	3.02	3.02	.	0.45	0.45	.	1.7
B. rapidum (LeC.)	♀	1	4.17	4.17	.	1.02	1.02	.	4.3
Bradycellus rupestris (Say)	♀	17	4.28	4.26 ± 0.05 ns	3.9 - 4.7	0.89	0.90 ± 0.06 ns	0.26 - 1.23	3.4 ± 0.1
	♂	19	4.20	4.21 ± 0.04 ns	3.8 - 4.5	0.84	0.87 ± 0.09 ns	0.15 - 1.45	3.3 ± 0.1
B. tantillus (Dej.)	♀	13	2.91	2.86 ± 0.04 ns	2.6 - 3.1	0.37	0.35 ± 0.02 ns	0.19 - 0.52	1.12 ± 0.04
	♂	10	2.77	2.78 ± 0.03 ns	2.6 - 2.9	0.35	0.33 ± 0.02 ns	0.17 - 0.43	1.02 ± 0.03
Calathus opaculus LeC.	♀	5	9.55	9.46 ± 0.17 ns	8.9 - 9.9	8.52	8.70 ± 1.01 ns	6.52 - 11.63	40.8 ± 3.4
	♂	4	8.69	8.73 ± 0.30 ns	8.0 - 9.5	6.40	6.47 ± 0.70 ns	5.19 - 7.89	30.9 ± 3.1
Calosoma scrutator (F.)	♀	1	35.82	35.82	.	377.50	377.50	.	3157.8
Chlaenius tricolor Dej.	♀	17	13.01	12.92 ± 0.10 a	12.1 - 13.6	23.51	22.49 ± 1.82 ns	10.74 - 34.48	132.2 ± 3.0
	♂	7	12.44	12.40 ± 0.22 b	11.5 - 13.2	11.19	14.97 ± 4.24 ns	3.38 - 33.46	98.7 ± 8.4
Cicindela punctulata Oliv.	♀	3	12.01	11.99 ± 0.05	11.9 - 12.1	17.03	16.92 ± 1.02	15.10 - 18.62	107.5 ± 2.6
	♂	1	10.83	10.83	.	15.84	15.84	.	53.7
Clivina bipustulata (F.)	♀	2	6.38	6.38 ± 0.39 ns	6.0 - 6.8	3.65	3.65 ± 0.97 ns	2.68 - 4.62	9.6 ± 1.2
	♂	1	6.45	6.45 ns	.	3.64	3.64 ns	.	10.2
Dicaelus elongatus Bon.	♀	1	15.59	15.59	.	49.16	49.16	.	188.2
	♂	2	16.82	16.82 ± 0.10	16.7 - 16.9	55.80	55.80 ± 0.61	55.19 - 56.41	259.6 ± 4.0
Dyschiriodes globulosus (Say)	♀	1	2.51	2.51	.	0.25	0.25	.	0.93
Elaphropus anceps (LeC.)	♀	12	2.35	2.32 ± 0.03 ns	2.1 - 2.5	0.14	0.15 ± 0.02 ns	0.07 - 0.30	0.68 ± 0.03
	♂	5	2.35	2.31 ± 0.06 ns	2.1 - 2.5	0.20	0.20 ± 0.02 ns	0.15 - 0.24	0.69 ± 0.02
E. xanthopus (Dej.)	♀	24	2.23	2.20 ± 0.02 ns	1.9 - 2.3	0.22	0.21 ± 0.01 ns	0.08 - 0.26	0.74 ± 0.02
	♂	14	2.18	2.15 ± 0.03 ns	1.9 - 2.3	0.20	0.20 ± 0.01 ns	0.12 - 0.26	0.67 ± 0.03
Harpalus affinis (Schr.)	♂	1	9.40	9.40	.	19.59	19.59	.	47.4
H. longicollis LeC.	♀	1	12.44	12.44	.	14.23	14.23	.	97.0
	♂	1	13.99	13.99	.	35.47	35.47	.	137.3
H. pensylvanicus (DeG.)	♀	33	15.94	15.91 ± 0.15 a	14.1 - 17.6	37.90	41.92 ± 2.67 a	17.74 - 77.51	206.1 ± 6.5
	♂	33	15.01	15.04 ± 0.10 b	13.9 - 16.2	29.76	31.34 ± 1.81 b	12.27 - 54.18	169.5 ± 3.9

Appendix G. Adult carabid beetle body metrics by species and sex, Contined.

Species	Sex	n	Median Length (mm)	Mean Length ± SE (mm)[a]	Length Range (mm)	Median Weight (mg)	Mean Weight ± SE (mg)[a]	Weight Range (mg)	Body Volume ± SE (mm^3)
Harpalus somnulentus Dej.	♀	11	8.67	8.72 ± 0.22 ns	7.3 - 9.8	9.14	8.64 ± 1.43 ns	2.21 - 15.02	39.0 ± 3.0
	♂	17	8.76	8.72 ± 0.12 ns	7.6 - 9.8	8.03	7.83 ± 0.78 ns	3.55 - 13.11	34.5 ± 1.2
Notiophilus novemstriatus LeC.	♀	3	5.03	5.01 ± 0.20 ns	4.7 - 5.4	3.38	3.47 ± 0.31 ns	2.98 - 4.04	6.8 ± 0.4
	♂	5	4.95	4.97 ± 0.09 ns	4.8 - 5.2	2.80	2.70 ± 0.21 ns	2.10 - 3.13	6.3 ± 0.4
Paratachys sagax (Csy.)	♀	5	2.29	2.24 ± 0.05 ns	2.1 - 2.3	0.11	0.11 ± 0.01 ns	0.081 - 0.13	0.55 ± 0.03
	♂	4	2.09	2.11 ± 0.04 ns	2.1 - 2.2	0.091	0.090 ± 0.005 ns	0.078 - 0.10	0.50 ± 0.03
Patrobus longicornis (Say)	♀	1	11.94	11.94	.	24.74	24.74	.	116.7
	♂	1	11.97	11.97	.	17.54	17.54	.	86.6
Poecilus chalcites (Say)	♀	9	11.58	11.62 ± 0.29 ns	10.2 - 13.2	19.56	21.33 ± 2.40 ns	11.00 - 31.46	78.1 ± 6.0
	♂	4	11.15	11.07 ± 0.13 ns	10.7 - 11.3	18.70	19.18 ± 2.45 ns	13.92 - 25.40	73.2 ± 11.1
P. lucublandus (Say)	♂	2	13.27	13.27 ± 0.81	12.5 - 14.1	30.99	30.99 ± 6.05	24.94 - 37.04	117.5 ± 1.2
Polyderis laevis (Say)	♀	13	1.30	1.29 ± 0.02	1.2 - 1.4	0.030	0.029 ± 0.002	0.012 - 0.038	0.13 ± 0.01
	♂	1	1.33	1.33	.	0.031	0.031	.	0.10
Pterostichus atratus (Newm.)	♀	2	15.52	15.52 ± 0.19	15.3 - 15.7	77.46	77.46 ± 13.42	64.04 - 90.88	208.5 ± 4.0
Scarites quadriceps Chd.	♀	4	21.23	21.46 ± 0.86 ns	19.8 - 23.6	127.06	128.04 ± 22.68 ns	82.30 - 175.7	421.6 ± 69.5
	♂	13	21.10	21.14 ± 0.33 ns	19.4 - 23.5	114.50	115.07 ± 6.48 ns	78.12 - 153.0	384.0 ± 22.4
S. subterraneus F.	♀	7	17.02	16.12 ± 0.77 ns	12.5 - 18.0	56.05	43.58 ± 10.20 ns	9.55 - 73.53	196.2 ± 27.4
	♂	12	16.78	16.28 ± 0.46 ns	13.5 - 18.6	58.19	57.12 ± 6.16 ns	18.11 - 87.10	192.5 ± 15.6
Selenophorus opalinus (LeC.)	♀	1	9.20	9.20	.	7.81	7.81	.	41.0
S. pedicularius Dej.	♀	1	6.13	6.13	.	1.67	1.67	.	12.5
Stenolophus conjunctus (Say)	♀	74	3.86	3.86 ± 0.03 a	3.3 - 4.8	0.77	0.81 ± 0.02 a	0.31 - 1.38	3.3 ± 0.1
	♂	56	3.72	3.70 ± 0.02 b	3.3 - 4.1	0.72	0.70 ± 0.03 b	0.25 - 1.16	2.9 ± 0.1
S. ochropezus (Say)	♀	34	5.91	5.87 ± 0.05 a	5.4 - 6.5	2.62	2.63 ± 0.15 a	0.85 - 4.57	10.7 ± 0.3
	♂	22	5.67	5.67 ± 0.04 b	5.3 - 6.1	2.14	2.00 ± 0.13 b	0.95 - 2.96	9.7 ± 0.3
S. rotundatus LeC.	♀	18	3.99	3.95 ± 0.06 ns	3.4 - 4.3	1.27	1.25 ± 0.08 ns	0.45 - 1.73	3.3 ± 0.1
	♂	19	3.98	3.98 ± 0.05 ns	3.5 - 4.3	1.21	1.11 ± 0.07 ns	0.53 - 1.65	3.1 ± 0.1
Tetracha virginica (L.)	♀	8	19.96	19.52 ± 0.54 ns	16.9 - 21.5	87.69	86.71 ± 3.47 a	73.49 - 97.96	436.5 ± 25.2
	♂	2	18.55	18.55 ± 0.09 ns	18.5 - 18.6	66.61	66.61 ± 4.26 b	62.35 - 70.88	345.8 ± 29.6

Appendix G. Adult carabid beetle body metrics by species and sex, Contined.

Species	Sex	n	Median Length (mm)	Mean Length ± SE (mm)[a]	Length Range (mm)	Median Weight (mg)	Mean Weight ± SE (mg)[a]	Weight Range (mg)	Body Volume ± SE (mm^3)
Trechus quadristriatus (Schr.)	♀	2	3.69	3.69 ± 0.02 b	3.7 - 3.7	0.62	0.62 ± 0.04 ns	0.58 - 0.66	2.6 ± 0.1
	♂	2	3.81	3.81 ± 0.01 a	3.8 - 3.8	0.67	0.67 ± 0.01 ns	0.66 - 0.67	2.9 ± 0.02
Trichotichnus fulgens (Csiki)	♀	11	6.68	6.66 ± 0.15 ns	6.0 - 7.3	4.81	5.12 ± 0.52 ns	2.64 - 9.09	17.8 ± 1.0
	♂	12	6.49	6.48 ± 0.09 ns	6.0 - 6.9	4.27	4.22 ± 0.32 ns	2.53 - 5.61	15.2 ± 0.6

[a] body lengths or weights followed by "ns" between sexes within species were not significantly different ($P > 0.05$); for significant differences, see ANOVA table (Appendix H).

Appendix H. Analysis of variance table for significant differences in carabid body length or dry weight by species.

Species	Body Length			Dry Weight		
	ddf	***F***	***P***	**ddf**	***F***	***P***
Acupalpus partiarius (Say)	56	14.03	0.0004	56	8.86	0.005
Amara familiaris (Duft.)	65	8.98	0.0039		-	
Chlaenius tricolor Dej.	22	6.28	0.0201		-	
Harpalus pensylvanicus (DeG.)	64	22.94	<0.0001	64	10.78	0.002
Stenolophus conjunctus (Say)	128	16.76	<0.0001	128	9.39	0.003
Stenolophus ochropezus (Say)	54	8.47	0.0052	54	8.64	0.005
Tetracha virginica (L.)		-		8	7.27	0.027
Trechus quadristriatus (Schr.)	2	34.34	0.0279		-	

Appendix I. Number and percentage of total carabid adults collected for each sampling method by species and sex[a] .

Taxa Code[b]	Species	Pitfall Trapping								Litter Extraction (Vacuum & Berlese)							
		Canopy		Anthesis		Harvest			%	Canopy		Anthesis		Harvest			%
		♂	♀	♂	♀	♂	♀	Total	Total	♂	♀	♂	♀	♂	♀	Total	Total
Acr	*Acupalpus partiarius* (Say)	.	.	3	2	.	2	7	1.9	13	24	39	33	4	11	124	6.5
Acu	*A. pauperculus* Dej.	.	.	.	.	.	.	.	.	3	8	2	2	12	12	39	2.0
Ago	*Agonum octopunctatum* (F.)	1	.	.	1	.	.	2	0.5	2	2	.	.	1	.	5	0.3
Agp	*A. punctiforme* (Say)	.	2	.	.	.	.	2	0.5	2	2	.	2	8	5	19	1.0
Ame	*Amara aenea* (DeG.)	11	20	1	.	.	1	33	9.0	85	125	13	17	26	23	289	15.0
Amg	*A. angustata* (Say)	.	.	.	.	.	.	.	.	.	.	.	1	1	.	2	0.1
Amt	*A. anthobia* Vil. & Vil.	.	.	.	.	.	.	.	.	2	1	.	.	.	.	3	0.2
Amc	*A. cupreolata* Putz.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	<0.1
Amf	*A. familiaris* (Duft.)	4	9	1	.	.	.	14	3.8	136	119	12	11	48	51	377	19.6
Ami	*A. impuncticollis* (Say)	2	1	.	.	.	.	3	0.8	2	4	4	3	1	3	17	0.9
Ams	*Amphasia sericea* (Harris)	.	.	.	.	.	.	.	.	1	1	.	.	.	.	2	0.1
Anc	*Anisodactylus caenus* (Say)	.	.	.	.	.	.	.	.	1	1	.	.	.	.	2	0.1
Anr	*A. rusticus* (Say)	1	.	1	1	.	.	3	0.8	.	2	3	5	5	6	21	1.1
Ans	*A. sanctaecrucis* (F.)	.	1	2	2	.	.	5	1.4	1	1	1	1	2	2	8	0.4
Ban	*Badister notatus* Hald.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	1	<0.1
Bea	*Bembidion affine* Say	.	.	.	.	.	.	.	.	1	1	.	.	.	.	2	0.1
Ber	*B. rapidum* (LeC.)	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1	<0.1
Brr	*Bradycellus rupestris* (Say)	.	.	4	1	.	.	5	1.4	13	11	3	2	21	17	67	3.5
Brt	*B. tantillus* (Dej.)	.	.	1	1	.	.	2	0.5	1	.	2	6	5	6	20	1.0
Cao	*Calathus opaculus* LeC.	1	.	.	.	.	1	2	0.5	1	2	.	1	3	1	8	0.4
Cas	*Calosoma scrutator* (F.)	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	<0.1
Ctr	*Chlaenius tricolor* Dej.	.	2	6	12	1	2	23	6.3	.	.	.	1	.	.	1	<0.1
Cip	*Cicindela punctulata* Oliv.	.	.	1	1	.	2	4	1.1	.	.	.	.	.	.	.	.
Clb	*Clivina bipustulata* (F.)	1	1	.	.	.	.	2	0.5	.	.	.	.	.	.	.	.
Die	*Dicaelus elongatus* Bon.	1	1	1	.	.	.	3	0.8	.	.	.	.	.	.	.	.
Dyg	*Dyschiriodes globulosus* (Say)	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	<0.1
Ela	*Elaphropus anceps* (LeC.)	1	6	.	1	.	.	8	2.2	1	3	3	2	1	3	13	0.7
Elx	*E. xanthopus* (Dej.)	3	5	.	.	.	.	8	2.2	7	12	.	.	.	3	22	1.2

Appendix I. Number and percentage of total carabid adults collected for each sampling method by species and sex, Continued[a].

Taxa Code[b]	Species	Pitfall Trapping								Litter Extraction (Vacuum & Berlese)							
		Canopy		Anthesis		Harvest			%	Canopy		Anthesis		Harvest			%
		♂	♀	♂	♀	♂	♀	Total	Total	♂	♀	♂	♀	♂	♀	Total	Total
Haa	*Harpalus affinis* (Schr.)	.	.	.	.	.	.	.	.	.	.	1	.	.	.	1	<0.1
Hal	*H. longicollis* LeC.	.	.	1	1	.	.	2	0.5	.	.	.	.	.	.	.	.
Hap	*H. pensylvanicus* (DeG.)	.	.	17	22	30	62	131	35.6	.	.	7	1	4	2	14	0.7
Has	*H. somnulentus* Dej.	.	.	.	.	.	.	.	.	.	.	11	5	27	23	66	3.4
Non	*Notiophilus novemstriatus* LeC.	.	.	.	.	1	.	1	0.3	1	1	1	1	2	1	7	0.4
Pas	*Paratachys sagax* (Csy.)	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	<0.1
Pal	*Patrobus longicornis* (Say)	.	.	.	.	1	.	1	0.3	.	.	.	.	.	1	1	<0.1
Poc	*Poecilus chalcites* (Say)	1	6	1	2	.	.	10	2.7	1	.	1	.	1	1	4	0.2
Pol	*P. lucublandus* (Say)	.	.	.	.	.	.	.	.	.	.	.	.	2	.	2	0.1
Poa	*Polyderis laevis* (Say)	.	.	.	.	.	1	1	0.3	.	4	.	.	.	2	6	0.3
Pta	*Pterostichus atratus* (Newm.)	.	.	.	1	.	.	1	0.3	.	.	.	.	.	1	1	<0.1
Scq	*Scarites quadriceps* Chd.	12	2	.	2	.	.	16	4.4	.	.	1	.	.	.	1	<0.1
Scs	*S. subterraneus* F.	3	4	8	3	.	.	18	4.9	1	.	.	.	.	.	1	<0.1
Seo	*Selenophorus opalinus* (LeC.)	.	.	.	1	.	.	1	0.3	.	.	.	.	.	.	.	.
Sep	*S. pedicularius* Dej.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	<0.1
Stc	*Stenolophus conjunctus* (Say)	.	.	.	2	1	1	4	1.1	37	46	35	41	212	229	600	31.2
Sto	*S. ochropezus* (Say)	1	3	16	25	.	.	45	12.2	30	24	6	9	3	1	73	3.8
Str	*S. rotundatus* LeC.	.	.	.	.	.	.	.	.	2	.	23	29	2	5	61	3.2
Tev	*Tetracha virginica* (L.)	.	.	2	8	.	.	10	2.7	.	.	.	.	.	.	.	.
Trq	*Trechus quadristriatus* (Schr.)	.	.	.	.	.	.	.	.	.	.	.	.	2	2	4	0.2
Trf	*Trichotichnus fulgens* (Csiki)	.	.	.	.	1	.	1	0.3	1	2	6	4	8	10	31	1.6
	Abundance (♂ : ♀)[c]	106 (1:1.5)		155 (1:1.4)		107 (1:2.1)		368 (1:1.6)b		742 (1:1.2)		354 (1:1)		825 (1:1.1)		1921 (1:1.1)a	
	Species Richness	17		22		11		31 nominal		27		27		31		43 nominal	

[a] Cropping event abbreviations, Canopy: canopy close, Anthesis: pollen shed, Harvest: post harvest.

[b] Species abbreviations (Taxa Code) correspond to taxa codes depicted in redundancy analysis biplots.

[c] Male to female ratios followed by the same letter within row were not significantly different ($P > 0.05$).

Appendix J. Acquisition and preparation of no-choice feeding test items presented to adults of the ground beetle, *Elaphropus xanthopus* (Dejean), with References.

Test items of plant origin

Bluegrass seed. Bluegrass seeds, *Poa pratensis* L. (Poales: Poaceae) (Angiosperm Phylogeny Group 2003) were obtained commercially and soaked for 24 h in dH_2O at ambient room temperature prior to use.

Chickweed seed. Seeds from common chickweed, *Stellaria media* L. Villars, and lesser chickweed, *Stellaria media* ssp. *pallida* (Dumortier) Ascherson & Graebner (Caryophyllales: Caryophyllaceae), were collected in early May from a corn field prior to planting. The collection site was located at a commercial farm in Talbot County, MD.

Field corn: Cry34/35Ab1 Bt and non-Bt. Anthers and pollen were collected from field corn, *Zea mays* L. (Poales: Poaceae), for a Cry34/35Ab1 Bt hybrid and near non-Bt isoline variety (Dow AgroSciences, Indianapolis, IN). Anthers and pollen were collected into paper bags from corn tassels during anthesis, in plots at USDA-ARS-BARC. Anthers were separated from pollen using fine mesh, washed with dH_2O to remove unreleased pollen, and then stored at -20°C. Anthers and pollen were thawed to 25°C prior to use.

Test items of animal origin

Slugs. Slugs, *Deroceras* sp. (Gastropoda: Agriolimacidae), were manually collected from corn fields during early August at USDA-ARS-BARC, Beltsville, MD. Slug eggs were collected from corn fields prior to planting (mid-April) at a commercial farm in Queen Anne's county, MD. For the dead slug treatment, adults were frozen at -80°C for 30 min, then thawed to 25°C prior to use.

Roundworm adults. Hermaphroditic adults of the free living nematode, *Caenorhabditis briggsae* (Rhabditida; Rhabditidae) (wildtype strain AF16), were obtained from the laboratory of Dr. Eric Haag, University of Maryland, College Park, MD. Adults were washed from colony plates (containing *Escherichia coli*) using M9 buffer solution (Sulston and Hodgkin 1988), and transferred via pipette to Petri dishes. Hermaphroditic adults are identified as possessing vesicle shaped embryos internally, whereas adult males possess a horse-hoof shaped tail. Males were not used. Nematodes were visually sampled under a stereoscope using backlighting. Following experimentation, beetles were examined externally for the presence of "hitchhiking" nematodes, on which none were found.

Soil mites. Soil mite adults, *Pergalumna corrugis* (Jacot) (Sarcoptiformes: Galumnidae) were collected from litter bags deployed in late-July in corn fields at USDA-ARS-BARC, Beltsville, MD. Mites were collected from litter bags (onion bags containing corn leaf, silk, and husk material), deployed into corn fields for a minimum of 14 d. Upon collection, litter bags were transferred in large plastic bags in coolers to the laboratory, where mites were aspirated into propylene vials (LA-VIALS®, VL25H-CLR, LA Packaging / L&A Plastic Molding, Yorba Linda, CA), and then transferred with fine paint brushes into Petri dishes (50 mm x 9 mm; 351006, BD Falcon™ Tight-fit Lid Dish, Becton Dickenson Biosciences Discovery Labware, Bedford, MA). Mites were maintained in a Petri dish lined with dH_2O moistened filter paper, and supplied with granular baker's yeast, *Saccharomyces cerevisiae* (Saccharomycetales: Saccharomycetaceae) (YSC-2, Sigma-Aldrich Inc., St Louis, MO) as food. The colony Petri dish was enclosed in a resealable plastic bag and kept at ambient laboratory conditions. Mite eggs were obtained by dissecting females collected from litter bags. Female mites were slowly crushed between the flat side of a pair of tweezers and a probe fitted with a No. 1 pin. The exoskeleton of a crushed mite was then teased apart using a pair of probes, and eggs were transferred singly to Petri dishes using the probe tip. Pre-oviposited mite eggs appear as ovoid, smooth, and translucent white. Eggs filled the entire body cavity of all females dissected, including regions near mouthparts. Of three females surveyed during dissection, the mean (± SE) number of eggs per female was 12 ± 1 (range 4).

Predatory mite adults. Predatory mite adults, *Parasitus* sp. (Parasitiformes: Parasitidae) were collected from litter bags deployed in late-July in corn fields at USDA-ARS-BARC, Beltsville, MD. Litter bag material consisted of decomposing corn leaves. Litter bags were transferred in large plastic bags in coolers

to the laboratory, where adult mites were aspirated into polypropylene vials, and then transferred with fine paint brushes to Petri dishes. For the dead predatory mite treatment, adults were frozen at 0°C for 20 min, and then thawed to 25°C prior to use.

Collembola. Egg, juvenile, and adult stages of the collembolan, *Entomobrya intermedia* Brook (Collembola: Entomobryidae) were used in studies. Adult collembola were collected from litter bags deployed in corn fields at USDA-ARS-BARC, Beltsville, MD. Litter bags containing litter were transported to the laboratory in paper-in-plastic bags, and then shaken into deep, white plastic tubs. Adult collembola were captured from tubs by sucking them against a fine mesh screen affixed to the end of polytubing connected to a vacuum pump, one or two at a time. Adult collembola were then transferred to Petri dishes by inserting the tube tip into a partially open Petri dish with dry filter paper and turning off the vacuum. Adult collembola either jumped off the mesh tip immediately or were dislodged by flicking the polytube. Filter papers were then moistened, and dishes stored at 20°C, and used the same day. For the dead collembola treatment, adults were frozen at 0°C for 20 min., and then thawed to 25°C prior to use. Collembolan eggs and juveniles were obtained from colonies established using *E. intermedia* adults. Adults from field populations were aspirated into polypropylene vials, and then emptied into half-pint canning jars (Ball® half-pint wide mouth jars, Alltrista Corp., Muncie, IN), filled 1.5 cm deep with a 9:1 (wt:wt) mixture of plaster of Paris (calcium sulfate hemi-hydrate [$CaSO_4$, ½ H_2O]) and neutralized activated charcoal (Fisher Scientific, Rochester, NY). To provide moisture and food, distilled/deionized water and granular baker's yeast, *Saccharomyces cerevisiae* (Saccharomycetales: Saccharomycetaceae) (YSC-2, Sigma-Aldrich Inc., St Louis, MO), were added to jars as needed (Sims and Martin 1997). Colonies were kept under ambient laboratory temperatures, and moved to new jars weekly. *Entomobrya intermedia* eggs appear as spherical, smooth, and opaque white. Of 15 egg masses surveyed, the mean (± SE) was 20 ± 2 eggs (range 20). Collembolan eggs were collected from jars using blunt probes and transferred to Petri dishes. Eggs were held at 8°C prior to experiment. First instar juveniles were obtained by incubating eggs in tightly fitted Petri dishes with moist filter paper at room temperature.

Colorado potato beetle. Eggs of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) were obtained from the laboratory of Dr. Galen Dively (University of Maryland, College Park). Colorado potato beetle egg masses deposited on leaf disks were stored at 4.4°C prior to use. Eggs were transferred with metal probes to filter papers, and placed in their natural vertical orientation by the tacky adhesive at the base of each egg.

Southern corn rootworm. Eggs of the Southern Corn Rootworm, *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), were obtained from the USDA-ARS-BARC rearing facility in Beltsville, MD. Eggs were loosened from shipping material using dH_2O and pipetted to Petri dishes. First instar larvae were obtained by incubating *D. undecimpunctata howardi* eggs at 25°C.

Western corn rootworm. Eggs of the Western Corn Rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), were obtained from the USDA-ARS-NGIRL rearing facility in Brookings, SD. Eggs were loosened from shipping material using dH_2O and pipetted to Petri dishes. First instar larvae were obtained by incubating *D. virgifera virgifera* eggs at 25°C.

Black cutworm. Black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), eggs, and larval instars one and three, were obtained commercially (Benzon Research, Carlisle, PA.), and kept in shipping containers with meridic diet at 11°C prior to use.

European corn borer. Egg masses of the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), were obtained from the Insect Pathology Laboratory at Iowa State University, and stored at 4.4°C prior to use. Egg masses containing approximately eight eggs each (similar sized masses) were selected and transferred to filter papers using short bristle paint brushes.

Thief ants. Adults of the thief ant, *Solenopsis* (*Diplorhoptrum*) sp. (Hymenoptera: Formicidae), were aspirated from the soil surface in corn fields during late-July at USDA-ARS-BARC, Beltsville, MD. For the dead ant treatment, adults were frozen at -20°C for 20 min., and then thawed to 25°C prior to use.

Drosophila melanogaster. Eggs, larval instars two and three, and pupae of the vinegar fly, *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) (Meigen 1830) were used in studies. All stages were collected from laboratory colony vials. Egg and larval stages were flushed with dH_2O into deep Petri dishes with dry filter paper. To remove fly food contamination, eggs and larvae were successively washed in dH_2O and transferred to new Petri dishes. Larvae were transferred to test dishes using flexible tweezers; eggs were transferred with metal probes. Live larvae were placed in the center of Petri dishes to limit movement prior to carabid beetle introduction. Live larvae were held at 11.5°C until use. For the dead fly treatments, larvae were frozen at 0°C for 20 min., then thawed to 25°C prior to use. Early-stage fly pupae were teased from colony vials with blunt metal probes, rinsed in warm tap water, and then rinsed in dH_2O. Flies were obtained from the laboratory of Dr. Leslie Pick (University of Maryland, College Park).

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Appendix K. Maximum mandibular gape of adult *Elaphropus xanthopus* (Dejean).

The maximum mandibular gape of adult *E. xanthopus* was estimated to generally compare acceptance or rejection of no-choice food items. To prepare beetles for gape measure, the head of an insect mounting pin (No. 1) was dipped in hot wax, cooled for an instant, and then pressed against the elytra of live *E. xanthopus* adults. Pinned beetles were then oriented on a microscope stage allowing examination from a dorsal aspect. Gape was measured by inserting successively larger insect pins (No. 000-0) between the mandibles until the maximum gape was determined. Maximum gape was defined as the greatest distance between the apical points of the mandibles (as estimated by pin diameter), where beetles could open their mandibles just enough to disengage the insect pin. Pin diameter was measured using a caliper (Scherr-Tumico Industries, St. James, MN) and compared to information from the pin manufacturer (Morpho®/Monarch®, Pardubice, Czech Republic). Three adult *E. xanthopus* (1 male, 2 females) were measured. Consistent across individuals, ≈0.30 mm (No. 00 pin) approximated the maximum mandibular gape for *E. xanthopus* adults.

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