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DEPARTMENT OF ENTOMOLOGY

THE POSSIBILITY OF TROPHIC TRANSFER OF NEONICOTINOID
INSECTICIDES FROM CROP PLANTS TO *PTEROSTICHUS MELANARIUS*
(COLEOPTERA: CARABIDAE) VIA PREY

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ABSTRACT

1. Neonicotinoids are the newest class of insecticides and there is ongoing research about their effects on beneficial species, including natural enemies. Because of their prevalence and variety of application methods, there are many different potential routes of exposure to non-target insects. Ground beetle Pterostichus melanarius L. is an important polyphagous predator of agricultural ecosystems and previous research has shown that this and other species of ground beetles are susceptible to neonicotinoids.

2. I conducted a simple experiment to test if toxicity from thiamethoxam, one of the principal neonicotinoids, can be transferred from black cutworms (Agrotis ipsilon), a target pest, to P. melanarius, a non-target predator, through predation. Black cutworms suffered high mortality rates after ingesting thiamethoxam-treated corn seedlings, but no significant mortality differences were seen between experimental groups of P. melanarius that had fed on dead toxified cutworms and the control groups. However, some indications of sub-lethal effects were seen in P. melanarius that had fed on toxic prey, from which the beetles recovered after several hours.

3. I argue that, given the limitations of this experiment, these sub-lethal effects are relatively mild, and that neonicotinoid exposure through such indirect methods can be more detrimental, especially in field situations where there might be increased risk of predation. I also review literature about existing research on neonicotinoids and ground beetles, as well as literature on neonicotinoids' effect on other beneficial natural enemies, and further research needs in this area.

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Introduction

Since the commercialization of synthetic pesticides in the 1940's, arthropod pest control has faced continuing changes and challenges. DDT and other chlorinated hydrocarbons were among the first modern, synthetic broad-spectrum insecticides; other classes of insecticides such as organophosphates and carbamates soon followed. The initial success of these chemicals led to a shift in economic entomology towards chemical control, neglecting other types of control including cultural and biological control (Kogan 1998). However, growing awareness of the consequences of depending on these insecticides, including toxicity towards non-target species, led to a reevaluation of pesticide usage and the advent of integrated pest management (IPM), which seeks to combine chemical and biological pest control for more sustainable management. With IPM came a more ecological perspective, and there was increased consideration for the influence of pesticides on natural enemies, including both lethal and sub-lethal effects (Croft 1990).

Neonicotinoids are a new class of synthetic pesticides that, like nicotine, bind to the nicotinic acetylcholine receptor (nAChR), producing neurotoxic effects (Tomizawa and Casida 2004). They are the most recently introduced class of insecticide and are popular because of their high specificity toward insects, their systemic nature, and their effectiveness, especially against piercing-sucking insects (Tomizawa and Casida 2004). Another property of ecological significance is environmental persistence; neonicotinoids have variable soil half-lives of 148-1155 days for clothianidin, 25-43 days for thiamethoxam, and >1 year for imidacloprid (Cloyd and Bethke 2010). Application methods of neonicotinoids depend on the plant species and agroecosystem, and can include application of granules to the soil, drip irrigation, and spraying, as well as seed dressing, pelleting and painting onto the plant or plant parts (Jeschke and Nauen 2008). One of the largest uses for this group of insecticides is application to maize seed: almost all of the maize seed planted in North America is coated with neonicotinoid insecticides at an application rate of 0.25 to 1.25 mg ai/kernel, a notable exception being in organic farming, which comprises only 0.2% of total acreage (Krupke et al 2012).

Unlike nicotine, neonicotinoids have low affinity for vertebrate nicotinic acetylcholine receptors and a high affinity for insect ones. Specifically, neonicotinoids have a selectivity factor, which is the ratio of LD_{50} of mammals over that of insects, of 456 compared to only 33 and 16 for organophosphates and methylcarbamates respectively (Tomizawa and Casida 2004). This selectivity owes to the unique structural features of the molecule, which follows a general pattern of an *N*-heterocyclylmethyl moiety, a heterocyclic or acyclic spacer, and an electronegative *N*-nitroimine, nitromethylene, or *N*-cyanoimine tip. There are seven major commercial neonicotinoids which consist of these three structural components and 12 unique substituents (Casida 2010). Neonicotinoids are metabolized in insects using certain enzymes, most prominently the cytochrome P450 mono-oxygenases (CYP450) enzymes, which are common in insects and vertebrates and function to detoxify foreign chemicals (Shi et al 2009). Some of the metabolites of neonicotinoids are more potent than the original compound; for example clothianidin, an ingredient in its own right, is among the primary metabolites of thiamethoxam. In fact, thiamethoxam is quickly converted to clothianidin in lepidopteran larvae and plants and has been described as a pro-insecticide whose effects are entirely attributable to clothianidin (Nauen et al 2003).

Natural enemies of pests are an important consideration for IPM, which seeks to understand and maximize the contribution of beneficial species to pest control. Generalist predators are beneficial species that can contribute greatly to pest control; a review of manipulative field studies showed that in ~75% of cases generalist predators reduced pest numbers significantly (Symondson et al 2002). Given IPM's focus on combining biological control agents (including natural enemies) with chemical controls, discerning selectivity of various insecticides against natural enemies is important. There are two modes of selectivity that are used in IPM to spare natural enemies: ecological selectivity and physiological selectivity (Morales-Ramos and Rojas, 2003). Ecological selectivity arises from differential exposure of pests and natural enemies in time and space through deliberate placement of pesticide or exploitation of phenological or behavioral differences. On the other hand, physiological selectivity arises from the differences in innate activity between pests and natural enemies in response to direct contact with a

pesticide (Hull and Beers, 1985; Metcalf, 1994). Ecological selectivity is more relevant than physiological selectivity for neonicotinoids; for example, systemic pesticides like neonicotinoids are usually more harmful to phytophagous pests that consume plant material, and in some cases are not harmful to entomophagous predators and parasites that feed upon pesticide-laden prey and hosts (Croft 1990). However, sometimes systemic insecticides are harmful to natural predators, either causing direct mortality or more subtle sub-lethal effects. Response to insecticides varies with the stage of natural enemies (larvae, nymph, or adult) and sometimes also varies from laboratory studies to greenhouse and field studies (Delbeke et al 1997; Studebaker and Kring 2003). Above ground predators might not be exposed to neonicotinoids applied as a drench or granule to the soil (Mizell and Sconyers 1992), but burrowing and below-ground predators may be exposed.

Natural predators can be exposed to insecticides in a variety of ways, including through direct, topical contact, contact with resides, ingestion of toxic prey/host tissues or ingestion or ingestion of toxic plant products (Ruberson et al 1998). Both foliar and granular applications of neonicotinoids can allow translocation of the insecticide into flowers and pollen, which presents a danger to beneficial non-target insects such as lacewings, honey bees, or other species that feed on nectar or pollen (Laurent and Rathahao 2003). Indirectly, use of neonicotinoids can negatively influence natural enemy populations by substantially decreasing the numbers of available prey or hosts. There are also indications of resistance of certain insect species to neonicotinoids, but the known incidences of resistance occur with pests rather than natural enemies (Rauch and Nauen, 2003).

Pterostichus melanarius (Illiger) (Coleoptera: Carabidae) is an important generalist predator in many ecosystems and has been studied extensively as a valuable predator in agricultural ecosystems (Bohan et al 2000, Symondson et al 2002). Previous laboratory tests have investigated the effects of older pesticides such as pyrethroids on adult *Pterostichus melanarius* (Mulligan et al 2006)*,* and one study has shown that the neonicotinoid component of transgenic treated maize seeds and contaminated pollen

causes mortality (Mullin et al 2005). The same study also mentions ingestion of toxified prey as a method of *P. melanarius* exposure to insecticides, but did not focus on this route of exposure as much as on direct routes (Mullin et al 2005). Like other members of the family Carabidae, *P. melanarius* is a polyphagous and an opportunist omnivore that feeds on almost any suitable prey, including seeds, caterpillars and to a lesser degree slugs (Bohan et al 2001); one experiment found the species *Pterostichus chalcites* Say and *Scarites substriatus* Haldeman to prefer dead black cutworms over live ones, indicating that ground beetles can also scavenge (Best and Beegle 1976). An acknowledged prey of ground beetles is the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), which is of interest because it is a major pest of many important crops including maize, *Zea mays* L. (Showers 1997). Since black cutworms do the most damage at early seedling stage, control methods are attractive if they are in place at planting and are low-maintenance, such as insecticidal seed treatments. A recent study found that low rates of clothianidin can provide additional control when combined with Bt corn, but on non-Bt corn clothianidin alone is not sufficient for control (Kullik et al 2011). The lab portion of this study found a positive relationship between cutworm mortality and rates of clothianidin, but low rates did not provide the expected mortality or feeding-deterrent effects (Kullik et al 2011). Neonicotinoid seed treatments of corn are extremely popular in the United States (Krupke et al 2012), so there is a high likelihood that black cutworms would interact with neonicotinoid-infused corn.

The research presented here investigates the influence of neonicotinoids, specifically the combined influence of thiamethoxam and its metabolite clothianidin, on *P. melanarius*, exploring the interaction between carabid species, the black cutworm *A. ipsilon,* and maize seedlings grown from thiamethoxam-treated seeds. Considering that neonicotinoids are toxic to other non-target insect species and that thiamethoxam and its metabolites have been found in plant and insect tissue, I hypothesized that cutworms will acquire thiamethoxam from their host plants, and then pass the insecticide to *P. melanarius* during predation, resulting in *P. melanarius* experiencing some level of poisoning. Based on research by Best and Beegle (1976) I also hypothesize that *P. melanarius* will readily eat both dead and live cutworms, broadening the risk of larvae

conveying insecticide to their predators. Consistent with previous research, I expected that cutworm mortality will be low following exposure to low rates of thiamethoxam but mortality will increase with the application of high rates of thiamethoxam (Kullik et al 2011). By focusing on the interaction between these prey and predator species in a laboratory setting I hoped to glean insights into broader issues of the toxicity of neonicotinoids to beneficial non-target insects. This experiment is hardly comprehensive, so I will also discuss future research needs as well as potential contribution of neonicotinoids to integrated and sustainable pest management.

Materials and Methods:

Plants and insects

For this experiment I used four types of corn seed treatments: untreated, treated with fungicide only (Apron® XL, Maxim® XL and Dynasty®; active ingredients: fludioxonil, azoxystrobin and mefenoxam), treated with the same fungicides and low thiamethoxam (LT; 0.25 mg/seed), and treated with fungicide and high thiamethoxam (HT; 1.25 mg/seed). All corn seeds were variety TA451-00 and non-GMO (T.A. Seed; Avis, PA). Black cutworms (*Agrotis ipsilon*) were purchased as eggs from Benzon Research (www.benzonresearch.com; Carlisle, PA) and grown to third or fourth instar in diet cups (room temperature, 14:10 L:D) prior to use in the experiment. Members of the Tooker Lab captured ground beetles (*Pterostichus melanarius)* from corn and soybean fields the summer and fall of 2012 and housed them individually in 16-oz plastic containers (Reynolds Del-Pak®) with a layer of moist potting soil. Tooker lab personnel fed the beetles kitten food (Purina® Pro Plan Selects) softened in water twice a week, and maintained them in a growth chamber (21°C, 14:10 L:D).

Preparation of corn plants

To evaluate the effects of seed-treatments directly on black cutworms and indirectly on ground beetles, I grew the four different types of corn treatments in a greenhouse (14:10 L:D, 27:22°C day:night) in square-shaped pots (4"x4" top, 3.75" deep) in general potting soil (insert soil info here) and watered the plants as needed. I planted the corn plants three

weeks before ordering the black cutworms to ensure that corn seedlings were at least at third leaf stage upon beginning the experiment. For a projected total of 200 cutworms based on the number of beetles and expected cutworm mortality from insecticide, I planted 400 corn seeds: 80 seeds each of untreated and fungicide, and 120 seeds each of low and high thiamethoxam. I planted four corn seeds in each pot and arranged the total of 100 pots in rows, with each row containing one type of treatment. I alternated these rows to randomize the amount of light the different treatments received. When the corn seedlings had reached two or three-leaf stage, I cut all suitable seedlings at the stem and harvested them. I placed each cut seedling into an Aqua Pick (www.michaelsfloralsupply.com) filled with de-ionized water to maintain plant turgor, and then placed seedlings in the Aqua Picks into 16-oz Reynolds Del-Pak® plastic containers with wet filter paper on the bottom.

Introduction of black cutworms to corn seedlings

To ensure that black cutworms were able to feed on the treated corn seedlings, I waited until most of the black cutworms had developed to the third and fourth instar, and then randomly assigned 60 to each of the insecticide treatments (LT and HT) and 40 each to the controls (untreated and fungicide). This arrangement was to account for the cutworm mortality rate due to neonicotinoids found in a previous study (Kullik et al, 2011). I placed the containers with corn seedlings and black cutworm in the growth chamber (21°C, 14:10 L:D) and observed them five days after the initial introduction, whereupon I made final observations on cutworm mortality and began the next phase of the experiment.

Introduction of dead black cutworm to beetles

The purpose of this portion of the experiment is to test the indirect effect of thiamethoxam seed treatments on ground beetles. Because of the limited number of *Pterostichus melanarius* remaining and the high mortality of black cutworms in the HT and LT groups, I only fed dead cutworms from the treatment groups to beetles (two dead cutworms per container and one beetle per container for HT and LT; n=9 in each group).

Live cutworms from the control groups were frozen for 30 minutes and then fed to the beetles (one caterpillar and one beetle per container because control cutworms were very large [6th-7th instar], n=13 and 14 for fungicide and untreated groups). I introduced the dead cutworms to the beetles in the late afternoon/evening and allowed the beetles to feed overnight; 24 hours after initial introduction I observed whether the caterpillars were eaten and beetle behavior, noting if beetles were alive or dead, their reaction when touched gently with the tip of tweezers, and burrowing ability (whether beetles were above or below ground after exposure to artificial or real light for at least 20 minutes). On the morning to noon of the next day (12-16 hours after the first set of observations) I again made and recorded observations on burrowing ability.

Statistical Methods

Data taken from this experiment was mainly categorical with small sample sizes, so I used the Fisher Exact Test to determine differences between treatments. I used the 2x2 Fisher Exact Test (Langsrud 2004) to assess for differences in the number of above- or below-ground beetles between experimental and control groups for both the first and second set of beetle observations. To test for differences in the number of uneaten and eaten cutworms as well as for differences in the number of dead/impaired and unimpaired beetles across the groups, I used a 2x4 Fisher Exact Test (only P_a, similar to p-value, is given) (Lowry 2013).

To compare the differences in cutworm mortality between experimental and control groups, I used the chi-square test of independence (2x2) (Ling 2008).

Results

Black cutworm mortality

Contrary to the results of a previous leaf tissue bioassay experiment with clothianidin (Kullik et al in 2011), mortality rates due to insecticide treatments were high, between 60-80% (Figure 1). Mortality was greatest in the high thiamethoxam group at 80% and slightly lower in the low thiamethoxam treatment group at 62%, but still higher than the expected 30% mortality (Kullik et al 2011). Difference in mortality was significant between treatment and control groups (all p-values effectively zero), but there was only a slight difference between mortality rates in HT and LT groups (p-value=0.0455). There were also a small percentage of cutworms in the HT and LT group that were unresponsive, i.e. alive but barely moving in response to stimulus. I assumed these cutworms to be in sub-optimal condition and possibly near death. As expected, fungicide and untreated control groups had negligible mortality of larvae, indicating that the fungicide does not appear toxic to black cutworms (Figures 1). Judging by the small amount of damage to corn seedlings at the experiment's end, larval deaths occurred relatively quickly within the five days of exposure.

Of the surviving larvae in the experimental groups, there were no apparent sub-lethal effects, as surviving larvae reached the sixth-seventh instar and were observed to be roughly as large as larvae in the control groups.

Pterostichus melanarius consumption of black cutworms

After the ground beetles were exposed for 24 hours to black cutworm carcasses, nearly all of the beetles had eaten at least one dead cutworm (Table 1). No statistical difference existed in the number of uneaten cutworms compared to eaten cutworms across treatments (P_a= 0.288). It is not clear what killed the single dead *P. melanarius* in the HT group, but I saw no evidence of predation on the cutworm. There were two dead (one left cutworm untouched) beetles and one heavily impaired (showing signs of paralysis) ground beetle in the LT group. However, I found no difference in the number of

alive/unimpaired vs. dead/impaired beetles among the control and experimental groups (Pa=0.422). On the other hand, sub-lethal effects, as measured by whether beetles had burrowed under the soil or remained on the surface, were apparent in both HT and LT groups of ground beetles the 24 hours after initial introduction of beetles and cutworms (Table 2 & 4) but faded by the following 12 hours (Table 3 & 5).

At 24 hours and 36 hours after initial introduction, most of the beetles in the control groups were underground, indicating the normal preferred state of the beetles. Significantly fewer beetles in the experimental groups were underground 24 hours after the initial introduction; this indicates sub-lethal effects, possibly confusion from alteration of perceptive abilities, from insecticide exposure, and is consistent with previous observations that sub-lethal doses of insecticides can cause increases in carabid activity (Lovei and Sunderland 1996).

Observations made 36h after introduction of the beetles showed a decrease in the severity of the indications of sub-lethal effects, and there was no significant difference in the location of beetles between experimental and control groups (Table 5).

Discussion:

The purpose of this experiment was to investigate the response of black cutworms to thiamethoxam seed treatments and then the response of the ground beetle *P. melanarius* to consumption of contaminated black cutworm carcasses. This last portion was done to assess if insecticide could be transferred from plant to target pest to non-target natural enemy via predation. No *P. melanarius* died after consuming the cutworms*,* but there were indications of sub-lethal effects, observed as an increased proportion of ground beetles above ground in experimental groups compared to that of control groups. This indication of sub-lethal effects faded within the final 12 hours of observation, but could indicate that exposed beetles had altered abilities to perceive light, increased irritation, or other neurological/sensory perturbations for several hours after exposure to sub-lethal amounts of insecticide (Desneux et al 2007). While the dangers of such

changes in behavior are not apparent from my experimental design, altered behavior caused by sub-lethal doses of imidacloprid can lead to increased vulnerability to predation in the field (Kunkel et al 2001). Limitations of this experiment include the short time in which the assessment occurred. Unlike a similar experiment testing ground beetle susceptibility to neonicotinoids which sustained the bioassay over 4 d (Mullin et al 2005), my experiment involved one incident of feeding to beetles, from which the beetles were able to recover in 36 h. Also, due to unexpectedly high mortality (Kunkel et al 2001), I did not feed any live cutworms to *P. melanarius.* Judging by the small amount of damage to corn seedlings and the shriveled look of the carcasses, cutworms in the high- and low-insecticide groups that I fed to beetles had died early on in the five days of feeding exposure to corn seedlings. There was therefore a slight time lag between cutworms dying from insecticide and their introduction to beetles, possibly diluting the potency of the insecticides. Moreover, *P. melanarius* did not show much aversion to caterpillars, consuming those from the experimental groups just as readily as those in control groups. This contrasts with previous work that placed seedlings, corn rootworms, and ground beetles together in microcosm setting, which showed a feeding deterrence and ground beetle mortality in plots with clothianidin-treated seedlings, with ground beetle exposure at least partly via ingestion of toxified prey (Mullin et al. 2005). Levels of insecticide exposure in this experiment may have been lower than those in previous works, in which food pellets were sprayed directly with imidacloprid at label and half-label rates (Kunkel et al. 2001). Considering the limitations of this experiment, as well as the results of similar studies, I can speculate that detrimental effects to *P. melanarius* beyond the ones seen here would be possible, had the exposure period to the toxified prey been longer and if levels of insecticide exposure had been more acute.

Black cutworm mortality rates observed in my experiment were inconsistent with rates in a similar laboratory study with Bt corn and clothianidin. The mortality rates for this experiment are 62% and 80% for low and high clothianidin, while the highest mortality rates in the previous experiment were 30% and 23% for non-Bt corn with high clothianidin and Bt corn with low clothianidin (Kullik et al 2011). Because I used lab protocols and materials similar to those of Kullik et al. (2011), it is not immediately clear

why the two assessments yielded different mortality rates. In the same study, field experiments contradicted the laboratory experiments by showing that Bt corn treatments, rather than non-Bt corn with high clothianidin, had the highest crop yields (Kullik et al 2011). The mortality rates that I observed are more consistent with those found in Mullins et al. (2005), where western corn rootworm in a corn seedling assay suffered mortality rates of 90% on the first day of exposure to clothianidin-treated seedlings. Additionally, the same experiment showed that after four days rootworms had lower mortality rates (40-60%) in microcosms with fungicide-treated seedlings, mortality which was entirely attributable to ground beetle predation (Mullin et al. 2005). Both my study and the Mullin et al. (2005) study indicate that neonicotinoid treatments are highly effective against larval pests in lab settings, but Kullik et al. offers the compelling field evidence that clothianidin applications are not as effective in the field as Bt corn at maintaining crop yields.

The context of my experiment involves an ongoing debate on the benefits and costs of neonicotinoid use, and their effect on natural enemies such as *P. melanarius* is just one aspect. Here follows a more detailed examination of the research progress and potential research areas, including discussions into the benefits of certain integrated pest management concepts, so as to better understand the situation.

To gain more knowledge about the effects of neonicotinoids on *P. melanarius* and to broaden research into the effects of neonicotinoids on ground beetles in general, a standardized range of bioassays with *P. melanarius* can be used. The International Organization for Biological Control recommends the following standard test types for testing pesticide selectivity against natural enemies: 1) laboratory, initial toxicity test 2) semifield, initial toxicity 3) semi-field, persistence 4) field tests (Croft 1990). Ideally, one study can cover many of these tests, such as one that tested rates of different insecticides on mortality of *Pterostichus cupreus*, using spray deposits on soil, direct sprays on the beetle (such as might be received by diurnal beetles), sprays on a vessel of soil and growing barley in which the beetle had been established for 24 hours (a simulated field exposure), treated food, and dry spray deposits on glass (Edwards et al 1984). For

neonicotinoids, most of the research and interest has been in their effects on bees, although there are compelling studies into their effects on important natural enemy insects as well (Desneux et al 2007; Cloyd and Bethke 2010). There have been even fewer studies into carabid natural enemies and neonicotinoids, but the existing studies cover diverse methods of exposure. For example, carabid beetles from 10 genera (*Agonum, Amara, Anisodactylus, Bembidion, Chlaenius, Harpalus, Patrobus, Poecilus, Pterostichus,* and *Scarites*) have been exposed to commercial neonicotinoids (imidacloprid, thiamethoxam, or clothianidin) via oral exposure (ingestion) to insecticide-treated seeds, pollen, and germinated seedlings, which constituted the laboratory portion of the assay, and finally the microcosm bioassay, in which beetles *S. quadriceps* Chaudoir and *H. pensylvanicus* DeGeer were caged with corn rootworm and treated corn seedlings (Mullin et al 2005). Carabid beetles have also been evaluated for sub-lethal effects, including changes in survival, behavior and fecundity, when exposed to imidacloprid via contaminated food pellets and direct spray, as well as residual toxicity on treated turf-grass (Kunkel et al 2001). This study is notable because it highlighted sub-lethal effects of a neonicotinoid, including the increased vulnerability to predation; however, these studies were conducted on a specific turf-grass environment and might not shed light on similar problems in agricultural ecosystems. My study with *P. melanarius* was focused on the potential for oral toxicity via contaminated prey, and there are many further experiments that one could logically pursue along the lines of standardized protocols to further define the ecosystem-wide effects of neonicotinoids on a particular natural predator. Given the long half-life of some neonicotinoids in soil, it is relevant to test if *P. melanarius*, a burrowing predator, can be intoxicated by neonicotinoid residue in the soil. It would also be relevant to conduct a microcosm experiment to observe whether *P. melanarius* suffers mortality and/or sub-lethal effects by ingesting plant material or prey, and if there are differences from live or dead ingested prey. In field studies, it is most important to assess first if existing populations of *P. melanarius* or similar carabid species can successfully control pest outbreaks, such as one of black cutworm, and then if neonicotinoids decrease carabid abundance and their capacity to control pests. One study found that seed treatments of neonicotinoids and

foliar application of pyrethroids with transgenic seeds affected the abundance of *H. pensylvanicus* and *P. melanarius,* possibly because early-season seed treatments caused mortality for carabid larvae (Leslie et al. 2009). And while results were only consistent for *H. pensylvanicus*, there is good indication that insecticidal seed treatments like neonicotinoids and foliar applications of pyrethroids, rather than transgenic corn, can directly influence *P. melanarius* populations in the field (Leslie et al. 2009).

There are numerous examples that *P. melanarius* can be an effective biocontrol agent (Lovei et al. 1996). Aphid populations have been shown to increase most rapidly in areas with low activity-density of *P. melanarius* (Winder et al. 2005). In potato ecosystems, *P. melanarius* preys on Colorado potato beetle larvae and eggs as well as aphids, with some individuals consuming up to 55 aphids and 24 Colorado potato beetle larvae in 24 hours; however, *P. melanarius* was less abundant in fields treated with aldicarb than in untreated fields (Alvarez et al. 2013). As a generalist rather than a specialist predator, *P. melanarius* has certain advantages over specialists. For example, despite low frequency of predation, generalists like *P. melanarius* can be more important predators than specialists due to their high population densities (Lukasiewicz 1996).

While *P. melanarius* can be an effective generalist predator, its presence alone is not sufficient to control outbreaks of a pest; it exerts stronger control when pest numbers rise slowly and its activity can prolong the period between outbreaks (Burn, 1992; Southwood and Comins, 1976). Unfortunately, the life cycle of *P. melanarius* does not always align with potential prey; adults appear most active in late July through August (Leslie et al 2009), while predation in spring and early summer is most important for reducing numbers of early-season pests, such aphids, cutworms and slugs, at population peak (Edwards et al. 1979; Chiverton 1986). The effectiveness of *P. melanarius* and other carabid natural enemies can be enhanced by habitat manipulation and by applying principles of conservation biological control, CBC, which can be defined as "modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms to reduce the effect of pests" (Eilenberg et al. 2001). CBC can be achieved with a variety of tactics, including habitat manipulation, and using

less insecticides or insecticides with restricted physiological or ecological selectivity to reduce mortality of natural enemies (Gurr et al. 2000). Habitat manipulation can provide natural enemies with resources, including physical refuge (Halaji et al. 2000). For example, raised earth ridges that are sown to perennial tussock-forming grasses, known as "beetle banks", can provide excellent over-wintering habitat for carabid predators, with overwintering densities equal to or greater than those in conventional fields (Thomas et al, 1991). Beetle banks can influence aphid densities through effects on the distribution of predators, and they have the potential to offer shelter and alternative prey for polyphagous predators (Collins et al. 2002). Habitat manipulation to enhance natural enemy effectiveness is strongly related to increasing plant diversity, as predicted by the enemies hypothesis, which argues that complex systems enhance predation by natural enemies; the enemies hypothesis, along with the resource concentration hypothesis, attempt to explain why pests attain higher populations in monoculture systems rather than diverse ones (Root 1973). This hypothesis has been supported by numerous studies (see review by Nicholls and Altieri, 2004), and because habitat manipulation for enhanced natural enemy capacity is considered an important component in pest management strategies in organic farming (Zehnder et al. 2007).

Neonicotinoids as a class of insecticides possess many advantages, as addressed in the Introduction. However, many of them are broad-spectrum insecticides (Elbert et al. 2008), and have unacceptable side-effects for many beneficial arthropods. The most prominent and controversial of the unwanted side effects is the harm to honey bees (*Apis mellifera*), which suffer sub-lethal alterations to bee neurobiology (Decourtye et al, 2004), and LD_{50} values as low as 0.0179 µg/bee for imidacloprid and 0.0218 µg/bee for clothianidin after topical application (Iwasa et al, 2004). Wide-spread use of neonicotinoids present numerous paths of exposure to bees: when grown from treated seeds, flowers of sunflower and maize contain on average 10 µg/kg imidacloprid, which is then partially moved to pollen at a few micrograms per kilogram; oral toxicity to bees can be around 40-60 µg/bee (Bonmatin et al. 2003; Suchail et al. 2001). Besides the well-documented risk to honey bees, neonicotinoids also detrimentally affect other beneficial insects. Third- and-fourth instar *Harmonia axyridis* Pallas are at risk for

neonicotinoid poisoning by feeding directly on treated corn seedlings; neurotoxic symptoms were observed in 72% of larvae, of which few recovered, and final mortality rates were 80% for clothianidin and 53% for thiamethoxam (Moser and Obrycki 2009). Imidacloprid exposure under laboratory conditions is toxic at field levels to several predator taxa, including species *Orius insidiosus* and *Chrysoperla carnea*; it is also toxic to *Hippodamia convergens* and *Geocoris* spp. at normal foliar application rates (Naranjo and Akey 2005; Elzen 2001; Mizell and Sconyers, 1992). In fact, compared to insect growth regulators, acetamiprid caused broader toxicity and is not recommended in initial stages of control where insecticide selectivity is crucial to a successful integrated control program (Naranjo and Akey 2005). Applications of neonicotinoids have the potential to disrupt whole ecosystems by their unwanted toxicity to natural enemies: a spider mite outbreak in New York City was caused by applications of imidacloprid, which was shown in lab studies to have poisoned predators of the spider mite *Tetranychus schoenei,* contributing to the elevated densities of spider mites (Szczepaniec et al., 2011). While not as widespread a phenomenon as toxicity to beneficial insects, resistance to neonicotinoids has also been documented with the Colorado potato beetle, *Leptinotarsa decemlineata*, and the whitefly*, Bermisia tabaci* (Rauch and Nauen, 2003); cross-tolerance has also been found in clones of green peach aphid *Myzus persicae* (Foster et al. 2003).

Given the known negative effects of neonicotinoids, as well as some affects that are still being studied, indiscriminate and blanket use of neonicotinoids is not ecologically sound. In fact, on 16 January 2013 the European Food Safety Authority explicitly listed the acute and sub-lethal risks of neonicotinoids to honey bees, stating that due to exposure from pollen and nectar "only uses on crops not attractive to honey bees were considered acceptable." Several European countries have reacted to these developments: France has suspended sunflower and corn treatments of imidacloprid, while foliar uses are allowed for other crops such as sugar beets and cereals. Germany, Italy, and Slovenia have temporarily suspended seed treatments of neonicotinoids, but foliar uses and sometimes seed treatments (in light of improved seed treatment and sowing methods) have been re-adopted (EPA 2013). Foliar application of neonicotinoids has not yet been implicated in honey bee toxicity, but it can be a source of toxicity to

natural enemies (Mizell and Sconyers 1992), and more research should be done to assess their influence on natural enemies after such partial bans. In terms of natural enemies, there are methods to mitigate the negative influence of neonicotinoid insecticides, including spatial separation by using spot treatments, which can be assisted by new technologies such as Geographic Positioning System (GPS) and variable rate technologies (VRT) hat control delivery rates in different parts of the field (Coll 2004). Other practices in mitigation for natural enemies include treatments of alternate rows (Hull et al, 1983), use of bait formulations that are attractive only to target pests, and the use of trap crops that attract pest populations away from the crop where they can be treated without disrupting natural enemies (Hokkanen, 1991).

In conclusion, the use of neonicotinoids can provide valuable control of insect pest species, but there influence on non-target arthropods results in controversy, and there is pressure to determine the extent of their negative effects on a variety of beneficial non-target arthropods, including natural enemies. As with other broad-spectrum pesticides introduced prior to neonicotinoids, they must be used judiciously. The current debate recalls the furor in the 1940s over DDT, and the warning that chemical insecticides "be used cautiously until their action on other forms of life and on the soil has been determined" (AAEE 1947, p. 149). While there has already conclusive research into the effects of neonicotinoids on some bee and natural enemy species, questions still remain. For instance, while natural enemies like *P. melanarius* may be exposed to lethal and sub-lethal doses of neonicotinoid via oral or contact exposure, the extent of exposure via contaminated soil and in field conditions is unclear. Within the field of natural enemy ecology, there are also many unknowns about the size of natural enemy populations needed to effectively control insect pests and prevent outbreaks (Collins et al 2002), or how to adopt tenets of habitat manipulation to enhance natural enemy effectiveness at field scales in diverse cropping systems (Zehnder et al 2007). Agriculture is progressing towards more ecologically sound pest management approaches, and research that contributes to our understanding of natural enemy biology and its interactions with crop management tactics will greatly increase our understanding and help guide the progress toward more sustainable methods.

Appendix

Table 1: Caterpillar and beetle interaction 24 hours post-introduction

	Cutworm uneaten	cutworm eaten	impaired	unimpaired	Dead
HT Pterostichus	3	6	0	8	1
LT Pterostichus	1	8	1	6	2
Fungicide Pterostichus	4	9	1	11	1
Untreated Pterostichus	1	13	0	13	1

Table 2: Number of above-ground and below-ground beetles observed 24 hours after initial introduction

	above ground	below ground
HT Pterostichus	6	2
LT Pterostichus	6	1
Fungicide Pterostichus	0	12
Untreated Pterostichus	1	12

Table 3: Number of above-ground, below-ground, and dead beetles following

	above ground	below ground	dead
HT Pterostichus	3	4	2
LT Pterostichus	3	4	2
Fungicide Pterostichus	1	10	2
Untreated Pterostichus	3	10	1

Table 4: p-values from Fisher Exact Test comparing above- and below-ground statuses for HT and LT beetles, 1st observations

	P-values
HT vs. untreated	0.004
HT vs. fungicide	0.0007
LT vs. untreated	0.002
LT vs. fungicide	0.002

Table 5: p-values from Fisher Exact Test comparing above- and below-ground statuses for HT and LT beetles, 2nd observations

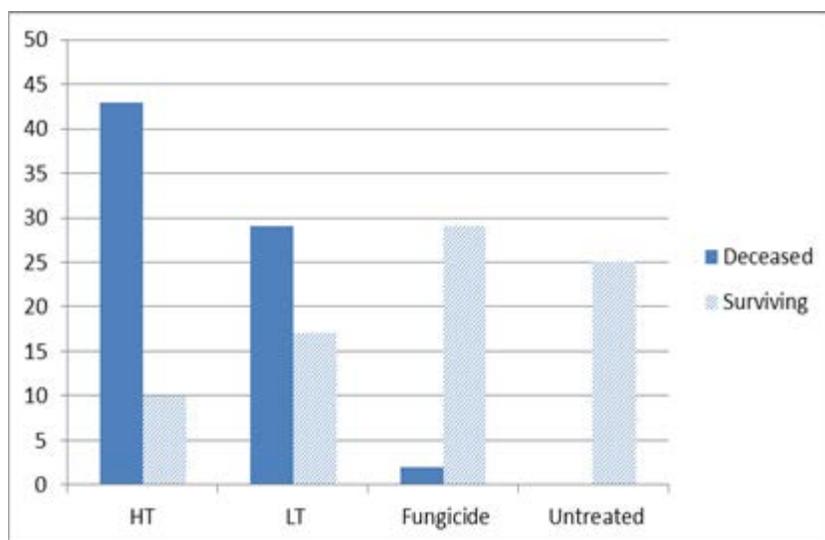|	P-values
HT vs. untreated	0.613
HT vs. fungicide	0.245
LT vs. untreated	0.613
LT vs. fungicide	0.245

Fig. 1. Number of deceased and surviving black cutworms after 120 h exposure to corn seedlings. HT=high thiamethoxam, LT=low thiamethoxam.

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Professional Experience

- Honors Independent Study, Tooker Insect Ecology Lab: September-December 2012

  As part of the research for my undergraduate thesis, I investigated the effects of

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  As an extern for the Bureau of Forestry, I completed a report on invasive species

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- Honors Independent Study, Cattadori Lab at the Center for Infectious Disease Dynamics,

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- Honors Independent Study, Gu Lab, Penn State University: May-July 2011

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