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GRANIVOROUS INVERTEBRATES AND WEED SEED PREDATION: AN ECOLOGICAL APPROACH TO WEED MANAGEMENT

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ABSTRACT

The objective of this dissertation is to better understand invertebrate agricultural weed seed predation and determine if predator olfaction and seed imbibition are important aspects of this interaction. Equally important, this project independently examines the use of a minimum tillage regime and as part of a larger integrated weed management strategy, including cover crops, to determine the influence of these management tactics on invertebrate activity-densities and weed seed predation. The minimum tillage regime did not reduce weed seed predation by invertebrates when compared to a no-till system. The majority of granivorous invertebrates had significantly higher activity-densities in minimum tillage plots when compared to no-till plots. However one species, *Harpalus pensylvanicus* DeGeer, an abundant seed predator, had a significant decrease in activity-density in min-till plots when compared to no-till. The integration of cover crops into a minimum tillage corn/soybean cropping system resulted in an increase in beneficial invertebrate activity-densities but did not increase surface seed predation when compared to no-till and organic systems.

Through a novel application of a stable isotope, ^{15}N, it was shown that *H. pensylvanicus* individuals frequently eat both velvetleaf (*Abutilon theophrasti* Medic) and giant foxtail (*Setaria faberi* Herrm) seeds in a no-till field. Imbibition of seeds altered *H. pensylvanicus* seed selection and the amount of seed eaten. All 7 weed seed species used in the study were eaten in significantly greater amounts when imbibed than when dry. Substantial amounts of large-sized seeds were eaten when imbibed but not when dry. Further illustrating the importance of imbibition, *H. pensylvanicus* individuals were shown to find imbibed velvetleaf (*Abutilon theophrasti* Medic) and giant foxtail *(Setaria faberi* Herrm) seed through olfaction but were unable to locate dry seed. Ethylene and carbon dioxide were released from dry seeds with greater

amounts of these two chemicals released when seeds were imbibed. However, *H. pensylvanicus* beetles showed no attraction response when exposed to these chemicals in y-tube olfactometry assays. This research shows that the application of a minimum tillage regime is not detrimental to the granivorous invertebrate community or seed predation when compared to a no-till system. The integration of cover crops into this tillage regime results in an increase in beneficial invertebrate activity-densities and does not decrease surface seed predation when compared to no-till and organic systems. Olfaction and seed imbibition are important aspects of *H. pensylvanicus* seed selection and predation.

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Chapter 1

Introduction

Agrestal weeds are estimated to cost around 33 billion dollars per year in crop losses, crop damage, and control throughout the United States (Pimentel et al. 2001). Traditionally, two methods have been used to reduce weeds in agriculture: tillage and the application of herbicides. Tillage was used exclusively until the first widely applied herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), was available in the late 1940's. Soon to follow was the production of the triazine group of herbicides produced in the 1950's. Since this time, the majority of growers have used both chemical and mechanical means to reduce weeds.

Traditional agricultural weed management, including tillage and herbicides, has been shown to have potential negative environmental consequences, both on and off the farm. Tillage is known to increase soil erosion by reducing soil residue cover and promoting soil organic matter degradation, thereby facilitating the loss of a valuable resource. The soil also often carries with it pollutants in the form of pesticides and nutrients to surrounding areas and waterways.

Due to the negative consequences associated with deep inversion tillage, a strong emphasis in corn-based cropping systems has been to move toward conservation tillage practices that reduce the soil erosion potential of these systems. Reduced tillage systems have been quite effective at reducing soil erosion and improving over-all soil quality, but this has come at the expense of heavy reliance on herbicides. Although herbicides such as glyphosate and the ALS-inhibitor herbicides are relatively environmentally benign, over reliance on these chemistries has led to the development of herbicide-resistant weed populations, rendering these weed control tools less effective. Many of the weeds common to the Northeast region, such as common lambsquarters (*Chenopodium album* L.), pigweed spp. (*Amaranthus spp.* L), common ragweed

(*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), yellow foxtail (*Setaria lutescens* Weigel), giant foxtail (*Setaria faberi* Herrm), and horseweed (*Conyza canadensis* L. (Crong.) have been shown to be resistant to one or more herbicide type (Heap 2012). Currently 330 resistant weed biotypes in 189 species have been identified in over 300,000 fields throughout the world (Heap 2012). In Pennsylvania, 7 resistant biotypes are known to exist (Heap 2012). Herbicide resistance has the potential to decrease crop yield and increase production costs.

Farmers also still rely heavily on residual pre-emergence herbicides in the triazine and chloraceamide families. These chemistries are commonly detected in surface and ground waters (EPA 2006, Gilliom 2007, Richardson 2006, USGS 2007) and concerns exist about the impacts of even very low levels of these pesticides on aquatic ecosystems (Berube et al. 2004, Hayes 2002). Herbicides are the most common pesticide found in both agricultural (atrazine, metolachlor, cyanazine, alachlor, and acetochlor) and urban (simazine, prometon, tebuthiuron, 2,4-D, and diuron) streams, with 56% of the 178 streams examined with developed aquifers having pesticide levels that exceeded EPA guidelines for aquatic life (Gilliom 2007).

Integrated Weed Management

Due to these environmental concerns, new integrated mechanical-chemical approaches to weed and crop management are being considered. In contrast to heavily relying on either herbicides or mechanical treatments, integrated weed management systems several control tactics to effectively control weeds with a focus on crop health. Such measures may include cover cropping, reduced tillage, crop rotation, etc. Individually these measures may not significantly reduce weed emergence but working in concert they can be effective. Numerous studies have found that post-planting mechanical weed control can substantially reduce the amount of herbicide applied and still effectively control weeds (Mulder and Doll 1993, Pleasant et al. 1994,

Buhler et al. 1995, Bates et al. 2012). However, it is also important to have reduced tillage throughout the growing season as this keeps weed seed closer to the surface, reduces seed persistence (Lutman et al. 2002), and allows for more effective control by herbicides due to earlier germination and emergence (Bullied et al. 2009). Weed seeds that are kept closer to the surface are also eaten in greater numbers by natural predators (Cromar et al. 1999).

For example, Bates et al. (2012) found that banded herbicide application coupled with select "surface tillage" operations (i.e. rotary harrow + high residue cultivation) provided cost-effective weed control without seriously compromising the soil conservation benefits of reduced tillage systems. This weed control was achieved with a 70% reduction in the herbicide active ingredient applied compared to conventional herbicide-intensive management. Although the integrated weed management systems outlined in Bates et al. (2012) show considerable promise to improve the sustainability of weed management systems in crops such as corn, they may also have negative consequences on management-based ecosystem services, such as seed predation.

Seed Predators

Seed predation can significantly impact herbaceous plant population dynamics (Abbott and Quink 1970, Reichman 1979, Crawley 2000) and in recent years, detailed investigations examining seed predation on weedy species in agricultural fields have been conducted (Cromar et al. 1999, Jacob et al. 2006, White et al. 2007, Saska 2008, Shearin et al. 2008, Westerman et al. 2008, Bohan et al. 2011, Ward et al. 2011, Baraibar et al. 2012). Ground-dwelling invertebrates have been found to be the dominate seed predators in some agricultural systems, accounting for as much as 80% to 90% of all seeds consumed (Cromar et al. 1999). The most important invertebrate seed predators in agricultural systems are ground beetles (Carabidae) with

populations estimated to consume as many as 1000 seeds m^{-2} day^{-1} (Honek et al. 2003) and crickets (Gryllidae) often the most abundant seed predators (O'Rourke et al. 2006).

Carabids are ground-dwelling insects that exist in a vast array of habitats spanning from tropical rainforests to semi-arid deserts (Holland 2002). This family is found in forest, grass, and agricultural ecosystems (Holland 2002). As many as 49 species of carabid beetle have been captured in a study in a temperate northeastern maize field (Leslie 2007). However, ten species accounted for the majority (>75%) of captures. Of these ten species, six are found within a genus known to consume weed seeds (i.e., *Harpalus pensylvanicus, Bembidion quadrimaculatum oppositum, Pterostichus melanarius, Chlaenius tricolor, Harpalus herbivagus,* and *Bembidon rapidium* (Holland 2002).

The most abundant species over the course of the 2 year study was *H. pensylvanicus* (Leslie 2007). *H. pensylvanicus* is found throughout the United States and is common in Pennsylvania. This species is mostly nocturnal and occupies a diverse range of habitats including forests, grasslands, pastures, and fields (Larochelle and Lariviere 2003). *H. pensylvanicus* breeds in the autumn and has the highest activity-density in maize fields in August and September (Leslie 2007, Ward et al. 2011). Individuals are rarely active before mid-July (Leslie 2007). Adults can survive two reproductive seasons but only one generation per year is produced. The majority of larvae emerge from pupae in late summer. *H. pensylvanicus* is a dominant weed seed predator (Westerman et al. 2008) and is believed to synchronize its breeding season with the release of grass weed seed (Holland 2002).

A number of other carabid beetle species are also capable of eating a variety of grass and broadleaf weed species (see Lundgren 2009). Likewise crickets have been shown to feed on a variety of grass and broadleaf weed seeds including *Chenopodium album, Amaranthus retroflexus, Setaria faberi,* and *Abutilon theophrastii* (Lundgren and Rosentrater 2007).

Greenhouse studies have shown a 50-60% reduction in broad-leaved weed seedling biomass with the presence of both carabid and cricket seed predators (Brust 1994). In addition to adult carabids consuming seeds, carabid larvae are known to consume and cache a variety of weed seeds (Kirk 1972, Hartke et al. 1998). First, second, and third instar larvae of members of the genus *Harpalus* have been shown, in a laboratory study, to eat all species of the ten most common weed seeds found in Maine (Hartke et al. 1998).

Seed Detection and Selection

Although much research has been done on carabids as seed predators, little work has examined how carabids detect seeds. Studies have shown that carabid carnivores use tactile, visual, and olfactory cues to detect prey (Toft and Bilde 2002). Nocturnal generalist insectivores of the *Pterostichus* genus use mainly olfactory cues detected by their antennae to find prey (Wheater 1989). However, it is unknown if granivorous beetles use these same cues to detect seeds. Knowing which senses are used in seed detection is important to help predict the degree of seed removal from the soil seed bank that can be expected by carabid beetles. For example, if beetles simply use visual, gustatory, and tactile means to find food and determine its palatability, visually obscured seed would often be left untouched. Alternatively, if beetles sense weed seeds through olfaction and can frequently find visually obscured seed, they would be a more effective component of an integrated weed management strategy and would greatly reduce selected species in the weed seed bank. Although no direct evidence for the use of olfaction in finding seeds has been reported, the indirect evidence available suggests that carabid beetles use olfactory capabilities in locating seeds.

Germinating seeds have been shown to release a number of volatile organic compounds, including carbon dioxide, alcohols, aldehydes, olefins, ketones, and volatile acids (Linton and

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Wright 1993), and germinating seeds were found to be preferentially consumed by both larvae and adults of the *Harpalus* genus when offered them amongst animal, plant, and fungal prey (Briggs 1965, Zhang 1993). White et al. (2007) found that seed predation of redroot pigweed (*Amaranthus retroflexus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti)* seeds by *H. pensylvanicus* did not differ between the soil surface and depths of 0.5 cm and 1 cm below the soil surface, suggesting that visually obscured seed can be located. Harrison et al. (2003) found that invertebrates, including carabids, preferentially choose to eat involucres that contain viable seed.

Although little is known about how invertebrates detect seed, it is well documented that many invertebrate seed predators exhibit weed seed preferences (Tooley and Brust 2002). The degree of seed selection varies between species and is thought to be affected by seed size and morphology (Brown et al. 1975, Inouye et al. 1980, Hartke et al. 1998), nutritional quality (Janzen 1969, Inouye et al. 1980, Hendry et al. 1994), and phenology (Honek et al. 2005). Selectivity in seed predation can alter weed community dynamics in agricultural fields. Brust and House (1988) documented a general preference for broadleaf weed species among carabids resulting in a significant shift in dominance toward grass species over a three year period. This was supported by White et al. (2007) who found that two carabid species (*H. pensylvanicus* and *Anisodactylus sanctaecrucis*) and the field cricket (*Gryllus pennsylvanicus*) preferred redroot pigweed seed (*Amaranthus retroflexus*) over giant foxtail seed (*Setaria faberi*). The work of Brust (1994) has also showed that selective feeding by carabids reduces the above ground biomass of broadleaf weed species, while other research has shown carabid preference for grass species (Lund and Turpin 1977, Saska 2008). Although no studies report total eradication of seeds by weed seed predators, it is quite apparent that through selective feeding and reduced biomass weed dynamics are greatly altered.

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One factor largely unaccounted for in seed preference studies is the role of imbibition. Of the numerous studies examining seed preference of carabids, only a select few have examined imbibed seeds (Cardina et al. 1996, Harrison et al. 2003). Both studies found imbibed seeds to be more accessible to carabid beetles. Cardina et al. (1996) found that the hard seed coat of velvetleaf was a deterrent to all invertebrate predators examined and only two predators would eat un-imbibed seed. Harrison et al. (2003) worked with imbibed smooth pigweed (*Amaranthus hybridus*), yellow foxtail (*Setaria lutescens*), and giant ragweed (*Ambrosia trifida*) after observing that dry ragweed seeds were not consumed by *H. pensylvanicus*. It seems likely that imbibition is an important factor in determining seeds palatability given that imbibed seeds have softer seed coats, are often metabolically active, and are frequently found in this condition in agricultural fields.

Although insightful, laboratory studies do not necessarily represent feeding preferences of study organisms in the field. Many carabid beetle species have been shown to eat weed seeds in a laboratory setting (Janzen 1969, Lund and Turpin 1977, Brust 1994, Honek et al. 2007, Lundgren and Rosentrater 2007, White et al. 2007) but these studies eliminate or reduce factors that can affect food selection including intraspecific and interspecific competition, habitat complexity and heterogeneity, and altering food availability and foraging costs. Most carabids are omnivores (Lundgren 2009) and understanding of their feeding habits in their natural environment is quite limited. Field studies often rely on correlation analyses to gain understanding of food webs. Field observations, stomach content analyses, and natural carbon and nitrogen stable isotope ratios provide a window into the diet of invertebrate species but are limited because they do not identify the seed species devoured (stomach content analysis and carbon and nitrogen stable isotope ratios) or are too difficult to sample to provide sufficient information about the population (field observations). Due to methodological shortcomings it has been difficult to determine invertebrate food selection in the field. In this work, I use a novel

application of isotopic enrichment of weed seeds to track species-specific invertebrate seed

selection and examine the role of imbibition in seed selection.

Effects of Disturbance on Seed Predation

In addition to negative impacts on both the chemical and physical soil properties of a

field, soil tillage alters the animal biodiversity within the field. A review study examining 106

publications found that the vast majority of taxa have a greater abundance or biomass in no-till

systems than conventional tilled systems (Wardle et al. 1995). Studies specifically examining

carabid beetles have found this trend to hold true. No-till systems have higher numbers of

carabid seed predators when compared to tilled organic and conventional systems (Cromar et al.

1999, Menalled et al. 2007). This is expected given that tilled systems can potentially result in

direct mortality, degraded habitat, and a reduction in food availability. In addition, the removal

of surface organic matter decreases habitat availability (Ferguson et al. 1984). Menalled et al.

(2007) found that seed eating carabids composed 32% of individual carabid captures in a no-till

system, 10% in an organic system, and 4% in a conventional system. In no-till systems, both

carabid diversity and evenness were twice that of conventional fields. A strong correlation

existed between the number of captured seed predators and the rate of seed predation for all

systems (Menalled et al. 2007). In a study of the direct mortality of carabids following tilling, it

was found that rotary tillage and moldboard plowing reduced weed seed predator activity-density

by 53% and 55% respectively (Shearin et al. 2007).

The timing of tillage has an effect on both the mortality of carabids and the availability of

seeds for predation. Autumn-breeding species, such as *H. pensylvanicus*, have been shown to

have a reduced population as a result of late spring tillage (Purvis and Fadl 2002). The beetle

larva in the soil can be killed mechanically, be adversely affected by the altered soil structure,

water flow, and temperature changes, and are more easily eaten by predators as a result of spring

tillage (Holland 2002). Fall tillage can result in mortality of adults and also buries seed that could

have served as a food source for granivorous populations. Since the life cycle of most crickets

and carabids takes a full year for completion and they produce only one generation per year, the

recovery from large scale disturbances is usually slow (Jepson and Thacker 1990). Little is

known, however, of the impact of low intensity tillage, such as that utilized in Bates et al. (2012),

on invertebrate seed predator activity and weed seed removal rates.

Effects of Cover Crops on Carabid Beetles

Cover-cropping is thought to aid in weed management by disturbing weed development

during the establishment of the cover crop, outcompeting weeds and inhibiting seed production,

and reducing seedling growth and establishment due to left over residues from the cover crop

(Sarrantonio and Gallandt 2003). Indirectly, cover crops may reduce weed establishment by

creating a microhabitat more beneficial for weed seed predators. Studies have shown that the use

of a red clover cover crop can increase the activity-density of carabid beetles (Comona and

Landis 1999) and can result in an increase in weed seed predation (Davis and Liebman 2003). In

Maine, *H. rufipes* had a higher activity-density in pea/oat-rye/vetch fields than fallow fields

(Shearin et al. 2008). It was also discovered that beetles released in fallow fields, in a mark-

recapture study, were more than twice as likely to be captured in pea/oat-rye/vetch fields than

vice versa (Shearin et al. 2008). However, some data has suggested that fall cover cropping can

be detrimental to seed predation (Gallandt et al. 2005) because fields are often plowed and

planted to the fall cover crop decreasing availability for weathering and predation of weed seeds

(Westerman et al. 2003).

Another benefit of cover crops comes from the increased soil surface residue after termination. In an agricultural setting, conventional field tillage removes all vegetation except for the crop or crops of interest. Creating this monoculture largely affects the quality and quantity of beneficial invertebrate habitat. However, it is possible to mitigate this lost territory to some extent. Conservation biocontrol is a form of biological control that seeks to increase the number of existing beneficial species in agricultural systems through habitat managment (Landis et al. 2000). Beetle banks are non-crop habitats established within fields that provide overwintering sites for carabid beetles (Thomas et al. 1991). Indeed, over 1100 carabids per m^2 were found to overwinter in these sites (Thomas et al. 1991). Other studies have corroborated this result and found significantly more carabid beetles overwintering in grassy strips or margins than bare ground or crop fields (Thomas and Marshall 1999). These areas are beneficial to carabids throughout the year. Non-crop habitats create a favorable microclimate, serve as shelter during extreme weather (Holland 2002), act as a favorable oviposition (Desender and Alderweireldt 1988) and aggregation site, and serve as a refuge during disturbance (Frampton et al. 1995).

Residues on the soil surface can be beneficial to carabid beetles in a way similar to beetle banks. Brust (1994) found that the combination of residue and seed predators greatly reduced weed growth. The incorporation of red clover residue into the soil resulted in a weed seed mortality similar to, but not greater than, exposure to weather, pathogens, and seed predators (Davis and Liebman 2003). Carabid habitat selection is governed by three factors: microhabitat, prey availability, and disturbance (Hance 2002, Thomas et al. 2002). Cover crops and their residue on the soil surface can greatly affect these three factors.

Objectives and Hypotheses

   The goal of this dissertation is to better understand invertebrate agricultural weed seed predation and determine if predator olfaction and seed imbibition are important aspects of the interaction. Equally important, this project examines the use of a minimum tillage regime independently and as part of a larger integrated weed management strategy, with the addition of cover crops, to determine the effects on invertebrate activity-densities and seed predation. Invertebrate seed predation is an important ecological and economic interaction in agroecosystems that has the potential to reduce farmers' reliance on herbicides through a reduction in weed pressure.

   In Chapter 2 the goal is to determine, when compared to a no tillage system, if a reduced tillage system will decrease weed seed predation by beneficial invertebrates and activity-density of the beneficial ground-dwelling invertebrate community. Throughout the literature it has been shown that a change to no-tillage cropping systems results in higher biomass for most taxa examined when compared to inversion tillage (Wardle et al. 1995). It is expected that the minimum tillage method to be employed will be similar to a no-till system by having minimal disturbance and will not affect the seed-eating carabid community. This minimum tillage method will only disturb the first 2-5cm of soil. It is expected that it will not directly kill carabid larvae in the soil, will not largely alter the soil structure or water flow, and will not lead to large temperature changes. Consequently, invertebrate activity-densities and seed predation in these systems will be similar to that of a no-till system. I hypothesize that granivorous invertebrates will not have a higher activity-density in no-tillage maize systems compared to minimum tillage (min-till) maize systems, and consequently invertebrate seed predation will not differ between these two tillage regimes.

In Chapter 3, I examine if the integration of cover crops into reduced tillage cropping systems will enhance the activity-density of the beneficial ground-dwelling invertebrate community, and thereby the pest management services with respect to weed seed predation. Cover crops create favorable habitats (Desender and Alderweireldt 1988, Holland 2002) and can act as aggregation sites for carabid beetles. Increased residue may also increase the amount of food sources other than weed seeds available to the omnivorous carabid predators allowing for a higher carrying capacity. I hypothesize that granivorous invertebrates will have higher activity-densities leading to increased weed seed predation in an integrated weed management system when compared to conventional no-till or organic systems due to reduced tillage (vs. organic) and increased surface residue (vs organic and no-till) from the cover crops and their remnants on the soil surface.

In Chapter 4, the goal is to determine if weed seed removed from the soil seed bank by invertebrate seed predators is weed species specific and if imbibition alters seed selection. I hypothesize that carabid beetle species, including *H. pensylvanicus* and *P. melanarius,* will eat both velvetleaf (*A. theophrasti*) and giant foxtail *(S. faberi)* seeds in a no-till field. In addition the role of imbibition on invertebrate seed selection will be evaluated. I hypothesize that the softer seed coat of imbibed seed will allow beetles access to larger seeds (i.e., ragweed (*A. artemisiifolia*) and velvetleaf (*A. theophrastii*) and these imbibed weed seed species will be eaten in greater numbers than when dry. Optimal foraging theory predicts that other things being equal, larger seed will be preferred as a means to maximize net energy intake (Schoener, 1971).

In Chapter 5, the objective is to determine if granivorous invertebrates use olfaction in detecting seeds. The first goal is to determine if volatile organic compounds are released by common weed seeds when dry or imbibed and if so, then to determine if *H. pensylvanicus,* a common weed seed predator known to prefer weed seeds as a food source (O'Rourke et al. 2006), would show a behavioral response to these compounds. All plants release volatile organic

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chemicals in response to changes in environmental parameters including light, nutrients, and water, and germinating seeds have been shown to release a number of volatile organic chemicals including CO_2, alcohols, aldehydes, olefins, ketones, and volatile acids (see Linton and Wright 1993). Carabid carnivores use olfactory cues to detect prey (Toft and Bilde 2002), thus it is expected that granivorous carabid beetles will similarly be able to sense and respond to olfactory cues. I hypothesize that the weed seeds examined will release volatile organic compounds and that *H. pensylvanicus* will show a behavioral response to the seeds themselves and the constituent chemical compounds released by weed seeds.

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Chapter 2

The Effects of Minimum Tillage on Invertebrate Weed Seed Predation

Introduction

Traditional agricultural weed management, including tillage and herbicides, can have negative environmental consequences, both on and off the farm. Tillage can increase soil erosion by reducing soil residue cover and promoting soil organic matter degradation, thereby facilitating the loss of a valuable resource. Eroding soil also often carries with it pollutants in the form of pesticides and nutrients to surrounding areas and waterways. Nearly 97% of the five million acres of corn planted in the United States receive herbicide treatment (NASS 2011). The primary concerns associated with herbicides are the contamination of water resources (EPA 2006), the potential negative impact on aquatic organisms (Bérubé et al. 2005), and the development of herbicide resistant weed populations (Heap 2012). Many of the weeds common to the Northeast region, such as common lambsquarters (*Chenopodium album* L.), pigweed spp. (*Amaranthus spp.* L), common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), yellow foxtail (*Setaria lutescens* Weigel), giant foxtail (*Setaria faberi* Herrm), and horseweed (*Conyza canadensis* L. (Cronq.)) have been shown to be resistant to one or more herbicide type (Heap 2012).

Due to these concerns, new integrated mechanical-chemical approaches to weed and crop management are being considered. For example, Bates et al. (2012) found that banded herbicide application in the row coupled with select "surface tillage" operations (i.e., vertical coulter + rotary harrow + high residue cultivation) between rows provided cost-effective weed control without seriously compromising the soil conservation benefits of reduced tillage systems. This weed control was achieved with a 70% reduction in the herbicide active ingredient applied

compared to conventional herbicide-intensive management. Although the integrated weed management systems outlined in Bates et al. (2012) show considerable promise to improve the sustainability of weed management systems in crops such as corn, they may also have negative consequences on management-based ecosystem services, such as seed predation.

Seed predation has been well documented to significantly impact herbaceous plant population dynamics (Abbott and Quink 1970, Reichman, 1979, Crawley 2000) and in recent years, detailed investigations examining seed predation on weedy species in agricultural fields have been conducted (Cromar et al. 1999, Jacob et al. 2006, White et al. 2007, Saska 2008, Shearin et al. 2008, Westerman et al. 2008). Ground-dwelling invertebrates have been found to be the dominate seed predators in some agricultural systems, accounting for as much as 80% to 90% of all seeds consumed (Cromar et al. 1999). Carabid beetles and crickets are the most important invertebrate seed predators in agricultural systems with carabid populations estimated to consume as many as 1000 seeds m^{-2} day^{-1} (Honek et al. 2003) and crickets found to be the most abundant seed predators (O'Rourke et al. 2006). A considerable body of literature exists on carabid beetle seed predation and it has been shown that a number of beetle species are capable of eating a variety of grass and broadleaf weed species (see Lundgren 2009). Likewise, crickets have been shown to feed on a variety of grass and broadleaf weed seeds including *C. album, A. retroflexus, S. faberi,* and *A. theophrastii* (Lundgren and Rosentrater 2007).

No-till cropping systems typically have higher numbers of seed predators compared to tilled (organic and conventional) cropping systems (Cromar et al. 1999, Menalled et al. 2007). Soil tillage likely impacts invertebrate seed predator numbers by causing direct mortality, habitat degradation, and a reduction of food availability through seed burial (Menalled et al. 2006, Shearin et al. 2008). Many carabid and cricket species life cycles take a full year for completion and produce only one generation per year. Consequently the recovery from large scale disturbances is typically slow (Jepson and Thacker 1990).

Little is known, however, of the impact of low intensity tillage, such as that utilized in Bates et al. (2012), on invertebrate seed predator activity and weed seed removal rates. Therefore, the objective of my study is to determine if the minimum tillage (min-till) regime employed in Bates et al. (2012) will decrease weed seed predation by beneficial invertebrates and to determine if the activity-densities of the beneficial ground-dwelling invertebrate community is reduced when compared to a no tillage (no-till) system. I hypothesize that seed-eating invertebrate activity-densities and weed seed predation will not be reduced in the min-till system when compared to a no-till system. The min-till method evaluated here disturbs the first 2cm-5cm of soil and is expected not to directly kill larvae in the soil, not largely alter the soil structure or water flow, and not lead to large temperature changes on the soil surface. Consequently, carabid beetle and cricket activity-densities in these systems would be similar to that of a no-till system. Seed-eating invertebrate activity-densities and weed seed predation rates will be compared between my min-till system and a no-till system. The depth of seed burial following min-till will be assessed.

Materials and Methods

Site Description

This study was conducted from July 6 to October 12, 2009 at the Russell E. Larson Agricultural Research Center in Rock Springs, PA located 16 km southwest of State College, Pennsylvania (Latitude: 40° 43' N Longitude: 77° 56' W). The effects of no tillage (no-till) and minimum tillage (min-till) on seed eating invertebrate activity-density and invertebrate weed seed predation were evaluated in a maize system. The minimum tillage utilized in this study replaces deep inversion tillage with field operations that retain residue on the soil surface while tilling shallow depths (less than 10cm). These tillage operations have previously been evaluated and

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compared to other reduced tillage measures in terms of surface residue cover, weed control, corn productivity and economic net returns (Bates et al. 2012). The fields utilized were predominately Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalf) and were in no-till maize production the previous year. The two fields utilized for experimentation were separated by 2.0 miles. Field 1 was 200m x 75m and consisted of 4 rows of paired 30m x 30m plots separated by 15m alleyways. This field consisted of 4 min-till plots and 4 no-till plots with a 7.5m corn buffer around the outside edges of the field. Field 2 was 244m x 60m and consisted of 5 rows of paired 30m x 24m plots separated by 15m alleyways. This field consisted of 5 min-till plots and 5 no-till plots with a 6m corn buffer around the outside edges of the field.

Crop Management

A standard corn variety for the region, Pioneer brand '36Y86', which contains glyphosate tolerance (Round Up Ready®) and lepidopteran resistance (Yield Guard®) traits, was planted. Two treatments, min-till and no-till, were applied on two separate fields that were in no-till corn production the previous year. The no-till treatments did not receive any mechanical weed control. These sites received a burndown herbicide program comprised of 0.84 kg ae ha-1 glyphosate [*N*-(phosphonomethyl)glycine] plus 0.28 kg ae ha-1 2,4-D low volatile ester [(2,4-dichlorophenoxy)acetic acid] and also received a pre-emergent residual herbicide program compromised of 1.87 kg ai ha-1 s-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide], 0.19 kg ai ha-1 mesotrione [2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione], and 1.54 kg ai ha-1 atrazine [6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine]. Post-emergence herbicides were later applied and included 0.027 kg ai ha-1 nicosulfuron [2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide], 0.0123 kg

ai ha-1 rimsulfuron [*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide], 0.84 kg ha-1 atrazine, and 0.28 kg ha-1 mesotrione. Herbicides were applied in water at 187 L ha-1 at 207 kPa.

The min-till treatment received the burndown herbicide program and also the application of a vertical coulter followed by a double pull rotary harrow on the day before planting. The vertical coulter has a series of fluted coulters that enter the first few centimeters of soil to provide increased air exchange and therefore increase soil temperatures. A vertical coulter is commercially available from Great Plains Mfg Inc. and is known as the Turbo-Till®. The vertical coulter does not provide any weed control but prepares the soil for increased activity by the rotary harrow. The rotary harrow has multiple rigid tines that uproot vegetation and therefore is useful as both a broadcast blind cultivator tool prior to planting and prepares the soil for planting (Gallagher et al. 2010). Post-emergent weed control was completed with two separate passes of a high residue inter-row cultivator in mid to late June at approximately the V6 growth stage (Ritchie et al. 2005) of the corn. The cultivator is designed to undercut weeds and leave residue on the soil surface.

Field Measurements

Two transects of six pitfall traps each were placed flush with the soil surface and perpendicular to the corn rows within each plot. Traps were placed to alternate between and within corn rows. Pitfall traps consisted of an outer 950 ml plastic container (height 9.4 cm x diameter 11.4 cm) that contained a 140 ml plastic collection cup filled with propylene glycol (insect killing agent). Inverted funnels created from 2-liter bottles separated mammals from the propylene glycol and funneled the specimens into collection cups. Trapping transects were placed 10m and 20m from the front edge of the plot. The trapping transect opened for data

collection was alternated each trapping session in order to avoid over-sampling an area of the field. Traps were placed 3m apart for a total transect length of 15m. Traps were open for a period of 72 hours twice a month from July thru Sept to assess the activity-density of carabid beetles in the two treatments. The following six carabid species were identified and recorded to species: *Harpalus pensylvanicus* DeGeer, *Bembidion quadrimaculatum oppositum* Say, *Pterostichus melanarius* Illiger, *Chlaenius tricolor* Dejean, *Harpulus herbivagus* Say, and *Bembidion rapidum* LeConte. These six species were the most abundant species found in crop fields in the area (Leslie et al. 2009) that are in a genus known to consume weed seeds (Tooley and Brust 2002). The total number of all other carabids not identified to species and crickets were also recorded.

Invertebrate predation of weed seeds was evaluated through the placement of seed cards set to coincide with the pitfall trapping sessions. Each seed card consisted of weed seeds placed on a cloth base surrounded by a vertebrate exclosure. *S. faberi*, *A. artemisiifolia,* and *A. theophrastii* seeds were placed on a 10cm x 10cm weed barrier cloth base at a density of 7500 seeds/m^2. This density is consistent with the natural density of seeds in fields of the area. The cloth base allowed for easy recovery of weed seeds not removed. Soil was placed on the weed cloth in order to make the substrate more similar to the natural soil surface. Corn residue was placed in and around the seed card in a manner consistent with residue levels in the area around the seed card. Exclosure cages, 10cm x 10cm x 12 cm (length x width x height), made of ¼" hardware cloth were placed over the seed cards to block access by vertebrates. Each seed card was placed a minimum of 5 meters away from other seed cards and the plot edge. Seed predation was quantified by counting remaining seeds after 168 hours in the field.

Seed burial by reduced tillage implements was assessed through the application and subsequent recovery of surrogate seeds. Surrogate seeds resembling true seeds in size and

density but composed of plastic and glass were used due to their high visibility and ease of recovery. Two sizes of surrogate seed were used to approximate the behavior of different sized seeds. The large surrogate seeds used were plastic and each weighed approximately .0292 grams and were the size of a velvetleaf seed while the small seeds weighed .0143 grams and were the size of giant foxtail seeds. Forty grams of small seed and 88 grams of large seed (approximately 3000 individual seed surrogates of both sizes) were randomly distributed in each of eight 2m x 2m plots on May 28, 2010. On this same day, four of these plots received the reduced tillage regime (vertical coulter, rotary harrow, and cultivator) with 20 soil cores taken between each tillage application. The other four plots received no tillage and served as no-till plots with surrogate seeds also being recovered via 20 soil cores per plot. Surrogate seeds were recovered from the soil cores and recorded to have been recovered at one of three depths: surface, 0-5cm, or 5-10cm.

Data Analysis

Generalized linear models (GLMs) were used to test for treatment effects and their interactions on the activity-density of invertebrates and for treatment effects on percent weed seed removal. Counts of invertebrate captures were used in data analysis of treatment effects. A repeated measures GLM model with a log-link and a Poisson error distribution was used for this analysis. Counts are bounded below since you cannot have a count below zero and therefore a log-link was used to lower bound the fitted values (Crawley 2007). The data were not normally distributed and, as such, a Poisson error distribution was used to account for this non-normality (Crawley 2007). Use of this statistical technique eliminates the need to transform data or use non-parametric analyses.

A repeated measures GLM was also used to test for treatment effects on percent seed removal data for three seed species (*S. faberi*, *A. artemisiifolia,* and *A. theophrastii*). A GLM model with a binomial error distribution was used for this analysis to account for the issues with using proportional data (boundedness, non-constant variance, and non-normal errors) (Crawley 2007). Likewise, this method eliminates the need to transform data or use non-parametric analyses. All data analyses were conducted in R.

Results

Community Composition

A total of 1,754 individual adult carabid beetles and 459 crickets were collected throughout the duration of the study. *H. pensylvanicus* and *P. melanarius* were the most abundant beetles captured accounting for 52.9% of all beetle captures. Temporal patterns of seed predator abundance varied (Figure 2-1). *H. pensylvanicus* activity-density peaked in late July to early August while *P. melanarius* activity-density peaked in late August to early September. *C. tricolor* had a consistently low number of captures throughout the sampling period with a slight increase in activity-density in the last trapping session that took place from October 8 through October 15. Crickets were captured in each trapping session with the highest activity-densities in the two trapping sessions in July. Carabid beetles, excluding the six granivorous species of high interest, had similar activity-densities across the sampling period (Figure 2-1). *H. herbivagus* and *B. rapidum* were excluded from analyses because too few individuals were captured.

Effects of Tillage

Four seed predator activity-densities (*P. melanarius, B. quadrimaulatum oppositum, C. tricolor*, and crickets) were significantly greater in min-till with only one species, *H. pensylvanicu,s* having significantly lower population in min-till plots when compared to no-till (GLM, $p < 0.001$)(Figures 2-1). No significant differences in activity-density were found in the group of unidentified carabid beetles between the two tillage regimes (GLM, $p > 0.05$)(Figures 2-1). The minimum tillage regime did not result in a decrease in seed predation (GLM, $p > 0.05$)(Figure 2-2). Seed predation did not differ between tillage treatments for any of the three seed species examined (GLM, $p > 0.05$). Predation, as estimated by seed loss from seed cards, ranged from $37.4 \pm 4.4\%$ to $97.3 \pm 0.7\%$ depending on plot type and date (Figure 2-2). Seed predation peaked in late July and was lowest in late September and early October for the 3 seed species and 2 tillage treatments (Figure 2-2).

In the no-till treatment, the majority of seed surrogates (beads) were recovered on the soil surface (Figure 2-3). Following all min-till implements (vertical coulter, rotary harrow, and cultivator), 57.5% of recovered beads remained on the soil surface (Figure 2-3). Small beads were more likely to be buried at any depth following all tillage implements with 56.4% found below the soil surface. In contrast, only 30.8% of large beads were found below the soil surface. Each successive tillage operation resulted in lower seed recovery at the soil surface, but similar recovery at the two burial depths.

Discussion

No-till farming is becoming more widespread due to the many benefits associated with it, such as improved soil quality, increased water infiltration and soil moisture, and reduced soil

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erosion (Lal et al. 1994). However concerns over herbicide resistance (Heap 2012) and pollution (Bérubé et al. 2005) that come as a result of extensive herbicide application in no-till exist. As such, the search continues for more sustainable and profitable methods to control weeds in agriculture. The results presented here show that the application of a min-till method, which provides effective mechanical weed control (Bates et al. 2012), has little detrimental effect on invertebrate seed predator activity and invertebrate seed predation. Although a complete consesus does not exist, it appears that extensive tillage in organic and conventional systems is deleterious to most granivorous carabid beetle populations. In a study of the direct mortality of carabids following tilling, rotary tillage and moldboard plowing reduced weed seed predator activity density by 53% and 55% respectively (Shearin et al. 2007). In addition, Menalled et al. (2007) found granivorous carabid species to be particularly vulnerable to tillage.

Our results show that min-till is not detrimental to granivorous invertebrate populations as deep inversion tillage appears to be. Min-till plots actually harbored more seed predators than no-till with twice as many *B. quad. oppositum* captured. Another known granivore, crickets, were also significantly more abundant in min-till plots. I believe that the application of min-till does not directly kill a significant number of invertebrate granivores. The redistributed surface residue, as a result of the min-till implements and, in particular, the high residue inter-row cultivator, created residue piles within corn rows that likely created a favorable micro-climate for beetles and crickets thus, leading to increased captures of these taxa. However a major seed predator, *H. pensylvanicus,* did have significantly lower activity-densities in min-till plots when compared to no-till. The reduction in activity-density in four of the six trapping sessions is possibly the result of decreased activity due to the surface residue piles created by the inter-row cultivator and not death or migration from plots. Alternatively, *H. pensylvanicus* individuals may have faced increased interspecific competition and emigrated from or been preyed upon in min-till plots. It is unlikely that mortality occurred as a result of direct effects from tilling as no other

species saw a decrease in activity-density. Future research using mark-recapture techniques would help to elucidate if *H. pensylvanicus* densities are reduced as a result of min-till or if this species simply has reduced activity in high residue systems.

Seed removal rates were not significantly affected by the application of a minimum tillage regime. This provides promise for employing this type of tillage as part of a larger IPM strategy. Seed predation was highest in July and August when invertebrate seed predators had the highest activity-densities. This suggests, as have other studies, that an increased number of invertebrate seed predators can result in a decrease in weed abundance (Gallandt et al. 2005, Westerman et al. 2005, Menalled et al. 2007). However, all the granivorous invertebrate species identified in this study peaked in activity-density before the peak release of most agricultural weed seed (*personal observation*). Some primarily carnivorous beetles (*Poecilus cupreus* L., *Dolichus halensis* Schaller, and *Synuchus vivalis* Illiger) feed on insects until seeds become widely available and then also eat seeds (Skuhravy 1959, Zhavoronkova 1969). It is unknown whether the highly granivorous but omnivorous beetles at the focus of this study exhibit the same pattern or if they continue to find and eat weed seeds from the previous year's crop of seed.

Given the apparent lack of synchronization between seed release of many weed species and beetle abundance, it seems likely min-till may bury a large proportion of the previous seasons weed seed. Over 42% of seed surrogates on the soil surface were buried through the application of the min-till regime. It is commonly believed that buried seed is not subject to predation by invertebrate granivores (Thompson 1987). However, few studies have examined weed seed predation at any soil depth. One such study by White et al. (2007) found that seed predation by *H. pensylvanicus*, one of the most abundant granivores in this study, on three types of seed was consistent at the soil surface down to 1cm below the surface (deepest depth examined). I am not aware of any studies examining invertebrate seed predation deeper than 1cm below the soil

surface. More research is needed to determine what effect seed burial has on predation by granivorous invertebrates.

It is possible that the min-till method applied here could lead to decreased seed predation and a higher density of seeds entering the seed bank. However, this method also likely moves seed from the soil seedbank to the soil surface where seeds are likely to have decreased persistance due to predation and weathering. In addition, soil disturbance can be an effective means to deplete the soil seed bank. Mulugeta and Stoltenberg (1997) found that seedling emergence in a high residue corn cropping system was 16 times greater in common lambsquarter, 6 times greater in giant foxtail, and 3 times greater in redroot pigweed following soil disturbance. Likewise, Roberts and Feast (1973) found a 32% decrease in viable seed following cultivation versus a 12% reduction in viable seed in undisturbed soil. It is then possible that addition seed entering the soil will be flushed out and controlled by subsequent tillage operations and not result in additional input to the seed bank.

Seed predation in my study was not weed species specific with all 3 seed species being eaten in near equal amounts. As such, neither broadleaf nor grass seed would have a higher likelihood of entering the seed bank based upon invertebrate predation. Previous laboratory and field studies have not found a consistent result for invertebrate seed preference with some showing preferences for broadfleaf weeds (Brust 1994) others showing a preference for grasses (Lund and Turpin 1977), and some, as is here, showing no preference for any weed seed type (Cromar et al. 1999). Alternatively, seed burial as a result of the min-till operations has the potential to affect seed bank composition. Nearly twice as many small seeds were buried as large seeds. This indicates that larger seeded species such as *A. artemisiifolia* and *A. theophrastii,* which increase in germination and emergence as a result of burial (Froud-Williams et al. 1984), will remain on the soil surface and be exposed to increase predation thus decreasing their presence in the soil seed bank in comparison to smaller seeded species.

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My study suggests that the application of min-till (i.e. vertical coulter + rotary harrow + high residue cultivation) does not result in a decrease in granivorous invertebrate activity-densities and does not decrease weed predation when compared to no-till. This min-till approach buries weed seed and could potentially obscure seeds from predators but is likely controlled via subsequent soil tillage operations. Min-till appears to be a promising option for the future that minimizes the environmental impacts of agriculture to the surrounding environment and does not hinder the ecosystem service of weed seed predation.
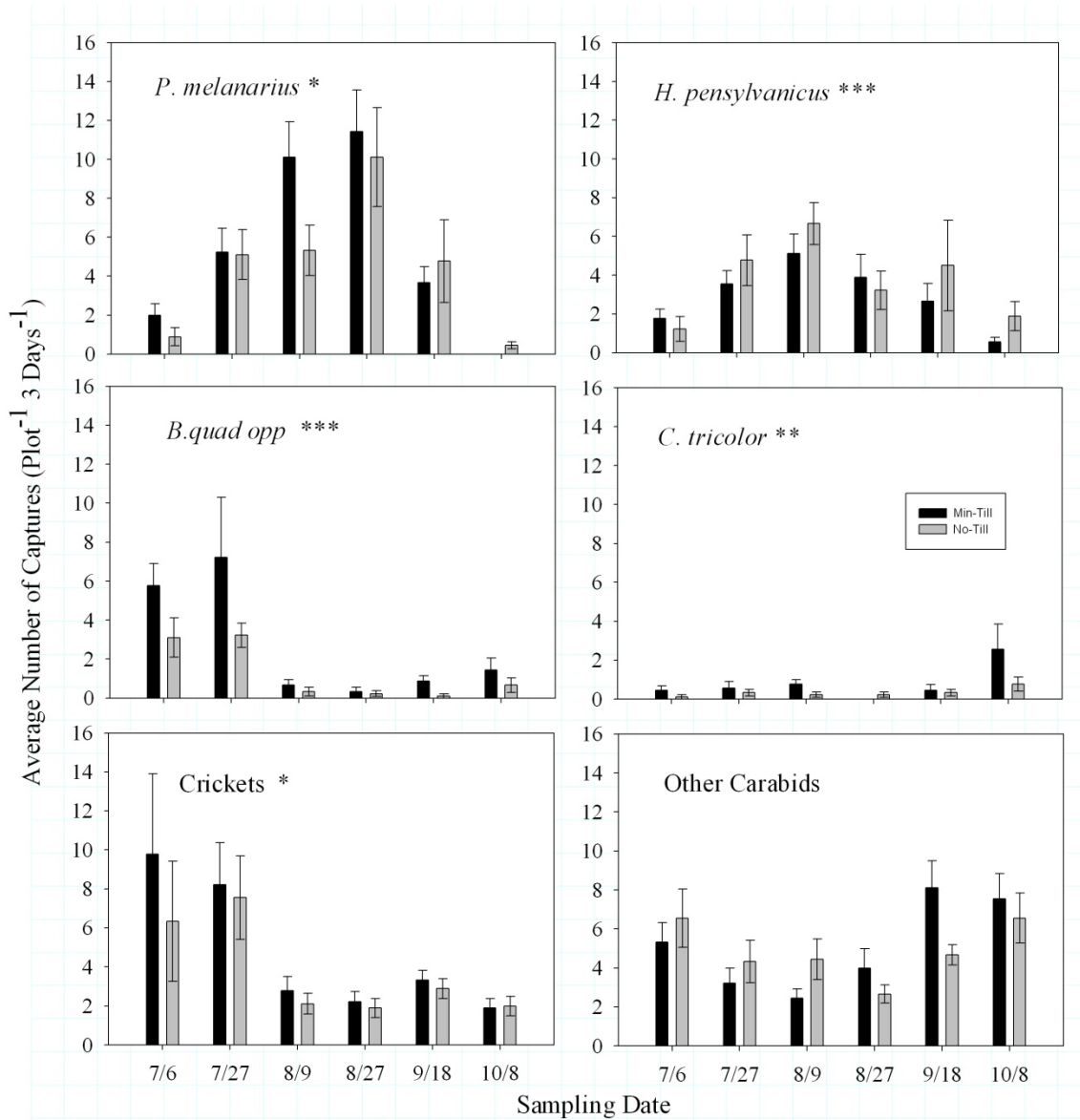
Figure **2-1**: Average number of individuals captured in no-till and min-till plots in 2009 at the Russel E. Larson Agricultural Research Center in Rock Springs, PA. Results shown for repeated measures GLM using poisson error distribution comparing activity-density between tillage treatments (min-till vs. no-till): *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Vertical bars represent ± standard error of the mean.
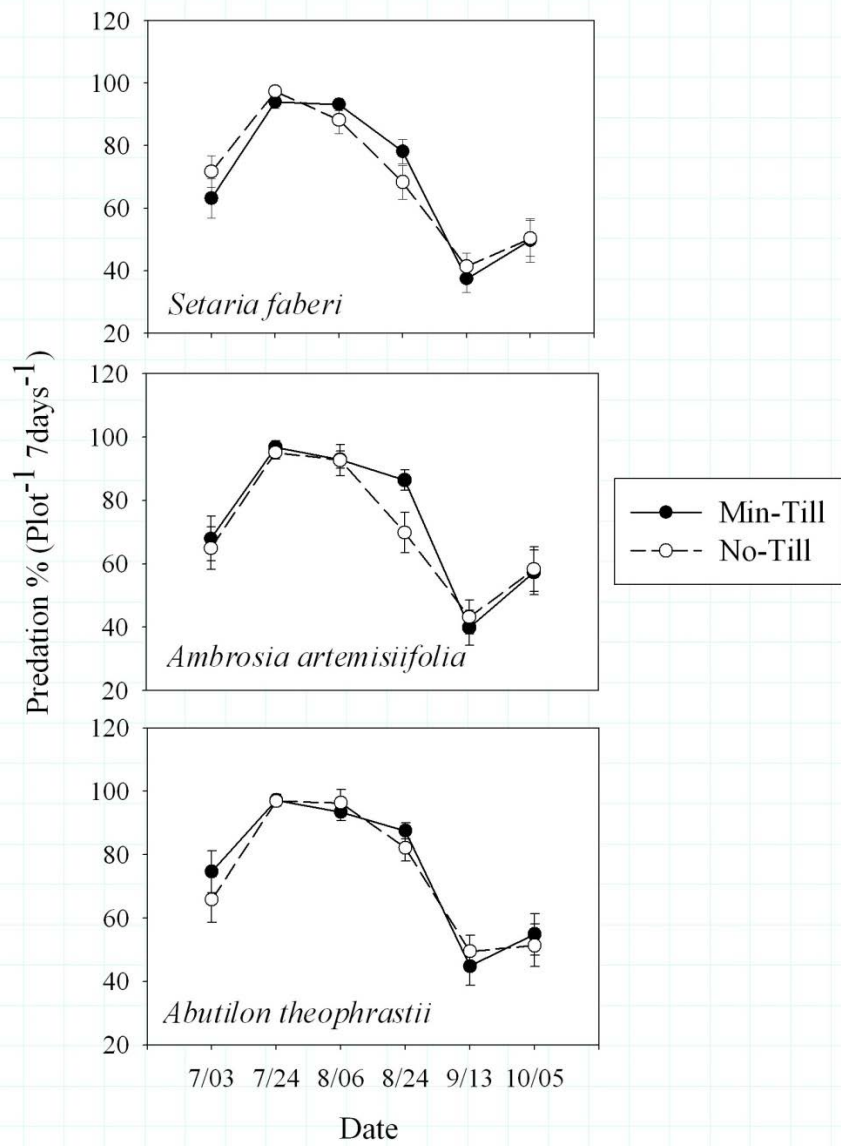
Figure **2-2**:   Percent seed predation of 3 seed species in no-till and min-till plots in 2009 at the Russel E. Larson Agricultural Research Center in Rock Springs, PA. No significant differences in predation percent were found between no-till and min-till for the three seed species. Vertical bars represent ± standard error of the mean.
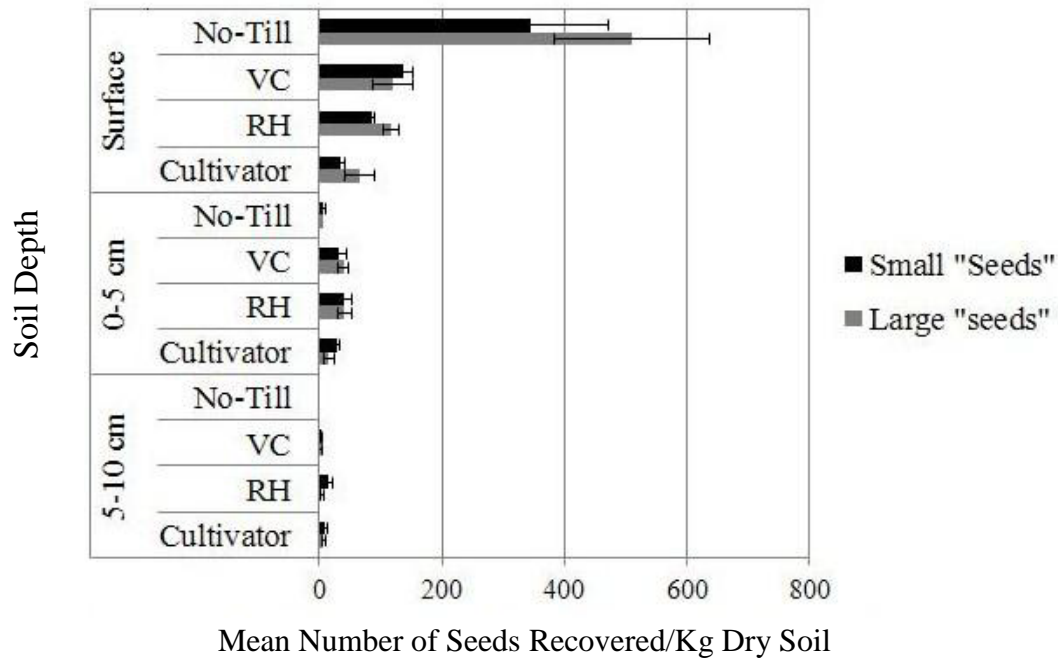
Figure **2-3:** Average number of small and large seed surrogates recovered at 3 different soil depths in no-till and following each successive tillage implement in min-till: VC = vertical coulter, RH = rotary harrow. Horizontal bars represent ± standard error of the mean.

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Chapter 3

Integrated Weed Management and Invertebrate Weed Seed Predation

Introduction

There has been a strong emphasis in corn-based cropping systems to move toward conservation tillage practices to reduce the soil erosion potential of these systems. Reduced tillage systems are known to reduce soil erosion and improve soil quality, but this benefit is at the expense of an over-reliance on herbicide application for weed control. Although some herbicides are thought to have a minimal environmental impact (i.e., glyphosate and the ALS-inhibitor herbicides) the frequent and widespread use of these chemistries has led to development of herbicide-resistant weed populations, resulting in reduced effectiveness in weed control. Farmers still rely heavily on residual pre-emergence herbicides in the triazine and chloraceamide families and these chemistries are commonly detected in surface and ground waters (EPA 2006, Richardson 2006, Gilliom 2007, USGS 2007), and concerns exist about the impacts of even very low levels of these pesticides on aquatic ecosystems (Hayes et al. 2002, Bérubé et al. 2005). Therefore, integrated weed and crop management systems are needed that adequately control weeds in conservation tillage systems.

A major factor that merits consideration in the development of an integrated strategy is how the weed control measures influence weed seed predation. Ground-dwelling invertebrates account for the majority of seed predation in some agricultural systems (Cromar et al. 1999). Carabid beetles (Coleoptera: Carabidae) and crickets (Orthoptera: Gryllidae) are the most important invertebrate seed predators in agricultural systems due to both their densities and the amount of seeds they consume (Honek et al. 2003, O'Rourke et al. 2006). Greenhouse studies

have shown a 50-60% reduction in broad-leaved weed seedling biomass with the presence of both carabid and cricket seed predators (Brust 1994).

Conventionally tilled soil preparation has many deleterious effects for an agricultural field.   This common practice has been shown to have negative impacts on both the chemical and physical soil properties. Conventionally tilled systems increase erosion, decrease organic matter, and increase soil surface temperatures. Consequently, the biodiversity of the system is largely altered. A review examining 106 publications found that the vast majority of taxa have a greater abundance or biomass in no-till systems than conventional tilled systems (Wardle et al. 1995).

Studies specifically examining carabid beetles have found this trend to hold true. No-till systems have higher numbers of carabid seed predators when compared to tilled organic and conventional systems (Cromar et al. 1999, Menalled et al. 2007). This is expected given that tilled systems can potentially result in direct mortality, degraded habitat, and a reduction in food availability. In addition, the removal of surface organic matter decreases habitat availability (Ferguson et al. 1984). Menalled et al. (2007) found that seed eating carabids composed 32% of individual carabid captures in a no-till system, 10% in an organic system, and 4% in a conventional system. In no-till systems, both carabid diversity and evenness were twice that of conventional fields. A strong correlation existed between the number of captured seed predators and the rate of seed predation for all systems (Menalled et al. 2007). In a study of the direct mortality of carabids following tilling, rotary tillage and moldboard plowing reduced weed seed predator activity density by 53% and 55% respectively (Shearin et al. 2007). Many carabid and cricket species life cycles take a full year for completion and produce only one generation per year. As such, population recovery from high mortality events, such as deep inversion tillage, is slow (Jepson and Thacker 1990).

However in contrast to deep inversion tillage, surface tillage effectively assists in weed control (Bates et al. 2012) while not being detrimental to invertebrate seed predators and weed

seed removal rates (Law, chapter 2). Bates et al. (2012) found that banded herbicide application in the row, along with "surface tillage" operations between rows, provided cost-effective weed control while conserving soil and reducing herbicide application. The tillage regime outlined in Bates et al. (2012) shows considerable promise to improve the sustainability of weed management systems in crops such as corn. In addition, this tillage regime only disturbs the upper 5 cm of soil and does not result in the invertebrate mortality observed with deep inversion tillage (Law, chapter 2). Weed seed is left near the soil surface where it is more prone to losses through predation and weathering. As such, surface tillage is likely an effective component of an integrated weed management strategy.

Cover-cropping aids in weed management by suppressing weed development during the establishment of the cover crop, outcompeting weeds and inhibiting seed production, and reducing seedling growth and establishment due to left over residues from the cover crop (Sarrantonio and Gallandt 2003). Indirectly, cover crops may reduce weed establishment by creating a microhabitat more beneficial for weed seed predators. Studies have shown that the use of a red clover cover crop increases the activity-density of carabid beetles (Comona and Landis 1999) and can result in an increase in weed seed predation (Davis and Liebman 2003). *Harpalus rufipes,* a granivorous carabid, had a higher activity-density in pea/oat-rye/vetch fields than fallow fields, and beetles released in fallow fields were more than twice as likely to be captured in pea/oat-rye/vetch fields than vice versa (Shearin et al. 2008).

Another benefit of cover crops comes from the increased soil surface residue after termination. The combination of residue and seed predators greatly reduces weed growth (Brust 1994). Both living and terminated cover crops have the potential to alter the three major factors affecting Carabid habitat selection: microhabitat, prey availability, and disturbance (Hance 2002, Thomas et al. 2002). The favorable habitat created by cover crops serves as shelter during

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extreme weather (Holland 2002), and act as a favorable oviposition and aggregation site (Desender and Alderweireldt 1988).

In this study, invertebrate activity-density and seed predation will be evaluated in an integrated weed management system that utilizes cover cropping in concert with surface tillage. This integrated system will be compared to a conventional no-till system and an organic transition system that utilizes cover crops. I hypothesize that the integration of legume green manure crops into a reduced tillage cropping system will enhance the activity-density of the granivorous ground-dwelling invertebrate community, and thereby the pest management services with respect to weed seed predation.

Materials and Methods

Site Description

This study was conducted from July 6 to September 24, 2010 and July 19 to October 12, 2011 at the Russell E. Larson Agricultural Research Center in Rock Springs, PA located 16 km southwest of State College, Pennsylvania (Latitude: 40° 43' N Longitude: 77° 56' W). The effects of integrated, no tillage (no-till), and organic transition (organic) cropping systems on seed eating invertebrate activity-density and invertebrate weed seed predation were evaluated in a maize/soybean system. The integrated system utilizes a minimum tillage regime that replaces deep inversion tillage with field operations that retain residue on the soil surface while tilling shallow depths (less than 10cm). These tillage operations have previously been evaluated and compared to other reduced tillage measures in terms of surface residue cover, weed control, corn productivity and economic net returns (Bates et al. 2012). The field utilized was predominately Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalf) and was in no-till barley

production the previous year. The systems (integrated, no-till and organic) were arranged in the field in a randomized complete block design with 4 replicates. Plots were 23m x 33m and blocks were separated by 15m alleyways. Originally a fourth plot in each block was a minimum tillage organic plot but this system proved too weedy to be an effective cropping system and was removed from the experiment.

Crop Management

2009-2010 Corn Crop

A hairy vetch (*Vicia villosa*) cover crop was planted in integrated and organic plots in the fall of 2009. Barley was grown in the field the previous season and barley residue is included in vetch biomass estimates. The vetch was chemically terminated before planting in May 2010 with a burndown herbicide program comprised of 0.84 kg ae ha-1 plus 0.28 kg ae ha-1 2,4-D in integrated plots. An organic untreated, non-gm corn variety, RPM N631, was then planted in all plots. However, seeds from the two non-organic plots, integrated and no-till, were treated with .249 kg Trace Latitude ® 100 kg-1 corn seed prior to planting. Latitude ® contains a neonicotinoid insecticide, imidacloprid, and two systematic fungicides, carboxin and metalaxyl. The no-till treatments did not receive any mechanical weed control but in addition to the burndown herbicide program, also received a pre-emergent residual herbicide program compromised of 1.87 kg ai ha-1 s-metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide], 0.19 kg ai ha-1 mesotrione [2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione], and 1.54 kg ai ha-1 atrazine [6-chloro-*N*-ethyl-*N′*-(1-methylethyl)-1,3,5-triazine-2,4-diamine]. Post-emergent herbicides were later applied and included 0.027 kg ai ha-1 nicosulfuron [2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide], 0.0123 kg

ai ha-1 rimsulfuron [*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide], 0.84 kg ha-1 atrazine, and 0.28 kg ha-1 mesotrione. Herbicides were applied in water at 187 L ha-1 at 207 kPa.

The integrated treatment received the application of a vertical coulter + double pull rotary harrow in place of a pre-emergent herbicide program prior to planting in May 2010. The vertical coulter has a series of fluted coulters that enter the first few centimeters of soil to provide increased air exchange and increase soil temperatures. A vertical coulter is commercially available from Great Plains Mfg Inc. and is known as the Turbo-Till®. The vertical coulter does not provide any weed control but prepares the soil for increased activity by the rotary harrow. The rotary harrow has multiple rigid tines that uproot vegetation and therefore is useful as both a broadcast blind cultivator tool prior to planting and prepares the soil for planting (Gallagher et al. 2010). Post-emergent weed control was completed with two separate passes of a high residue inter-row cultivator in mid to late June at approximately the V6 growth stage (Ritchie et al. 2005) of the corn. The cultivator is designed to undercut weeds and leave residue on the soil surface.

The organic treatment received no chemical weed control but instead received a series of mechanical tillage operations. Soil was prepared for planting by application of a moldboard plow, disk, harrow, and cultimulcher. Post-emergent weeds were controlled with two applications of a rotary hoe in early-mid June and subsequently, two applications of a cultivator.

2010-2011 Soybean Crop

Following corn harvest in early November 2010, winter rye was planted as a cover crop in organic and integrated plots. Rye was chemically terminated with 0.84 kg ae ha-1 plus 0.28 kg ae ha-1 2,4-D in integrated plots on May 25, 2011. An organic untreated, non-gm soybean seed variety, Blue River 34A7, was planted in all plots on May 31, 2011. However, seeds from the

two non-organic plots, integrated and no-till, were treated with .249 kg Trace Latitude ® 100 kg-1 prior to planting. Organic plots were controlled with the same mechanical applications as in 2010. No-till plots received a broadcast residual treatment of 1.42 kg ai ha-1 s-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]acetamide), 0.224 kg ai ha-1 metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one], and 0.039 kg ai ha-1 chlorimuron (2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid). Integrated plots also received this residual treatment but application was banded for in-row weed control. The planter was equipped with a small plot sprayer that applied a 30cm band of herbicide in corn rows. Herbicides were applied in water at 187 L ha-1 at 207 kPa.

Field Measurements

Invertebrate Sampling

Two transects of 6 pitfall traps each were placed flush with the soil surface and perpendicular to the corn rows within each plot. Traps were placed to alternate between and within corn rows. Pitfall traps consisted of an outer 950 ml plastic container (height 9.4 cm x diameter 11.4 cm) that contained a 140 ml plastic collection cup filled with propylene glycol (insect killing agent). Inverted funnels created from 2-liter bottles separated mammals from the propylene glycol and funneled the specimens into collection cups. Trapping transects were placed 10m and 20m from the front edge of the plot. The trapping transect opened for data collection was alternated each trapping session in order to avoid over-sampling an area of the field. Traps were placed 3m apart for a total transect length of 15m. Traps were open for a period of 72 hours from July thru September in 2010 and from July to October in 2011 in order to assess the activity-density of invertebrates in the treatments.

The following six carabid species were identified and recorded to species: *Harpalus pensylvanicus* DeGeer, *Bembidion quadrimaculatum oppositum* Say, *Pterostichus melanarius* Illiger, *Chlaenius tricolor* Dejean, *Harpulus herbivagus* Say, and *Bembidion rapidum* LeConte. These six species were the most abundant species found in crop fields in the area (Leslie et al. 2009) that are in a genus known to consume weed seeds (Tooley and Brust 2002). The total number of all other individuals belonging to the family Carabidae was also recorded. In addition, the number of individuals captured belonging to the family Gryllidae (crickets), order Opiliones (harvestmen), and order Aranea (spiders) were recorded.

Seed predation

Invertebrate predation of weed seeds was evaluated through the placement of seed cards set to coincide with the pitfall trapping sessions. Each seed card consisted of weed seeds placed on a cloth base surrounded by a vertebrate exclosure. *Setaria faberi*, *Ambrosia artemisiifolia*, and *Abutilon theophrastii* seeds were placed on a 10cm x 10cm weed barrier cloth base at a density of 7500 seeds/m^2. This density is consistent with the natural density of seeds in fields of the area. The cloth base allowed for easy recovery of weed seeds not removed. Soil was placed on the weed cloth in order to make the substrate more similar to the natural soil surface. Corn residue was placed in and around the seed card in a manner consistent with residue levels in the area around the seed card. Exclosure cages, 10cm x 10cm x 12 cm (length x width x height), made of ¼" hardware cloth were placed over the seed cards to block access by vertebrates. Each seed card was placed a minimum of 5 meters away from other seed cards and the plot edge. Seed predation was quantified by counting remaining seeds after 168 hours in the field.

Data Analysis

Repeated measures generalized linear models followed by Tukey's HSD post hoc tests on the best model were used to test for treatment effects on the activity-density of invertebrates and on percent weed seed removal. Model selection began with the null model and F-tests were used to compare models and determine if factors and their interactions increased model fit ($p \leq 0.05$). Counts of invertebrate captures were used in data analysis of treatment effects. A repeated measures GLM model with a log-link and a Poisson error distribution was used for this analysis. Counts are bounded below since you cannot have a count below zero and therefore a log-link was used to lower bound the fitted values (Crawley 2007). The data were not normally distributed and, as such, a Poisson error distribution was used to account for this non-normality (Crawley 2007). Use of this statistical technique eliminates the need to transform data or use non-parametric analyses.

Repeated measures generalized linear models were also used to test for treatment effects on percent seed removal data for three seed species (*S. faberi*, *A. artemisiifolia,* and *A. theophrastii*). A binomial error distribution was used for this analysis to account for the issues with using proportional data (boundedness, non-constant variance, and non-normal errors) (Crawley 2007). Likewise, this method eliminates the need to transform data or use non-parametric analyses.

Correlation of taxa abundance and seed predation was assessed using Spearman rank correlation analysis (Sokal and Rolf 1995). This is a non-parametric approach that is used to test for a monotonic rather than a linear association. Correlations were completed between each individual taxa and the combined percentage of all seeds devoured.

Analysis of variance (ANOVA) was used to test for treatment effects on crop yield in both 2010 and 2011. All data analyses were conducted in R (R: A Language and Environment for Statistical Computing 2011, R Development Core Team, Vienna, Austria).

Results

Agronomic

Hairy vetch averaged 9,794 kg ha^{-1} of dry matter across all plot types (Table 3-1). Corn yields in 2010 were statistically equivalent across treatments (one-way ANOVA, p-value = 0.653) averaging 9529 kg ha^{-1} at 15.5% moisture content with less than 5% of the surface area of all plots with weedy species (Table 3-1). The 2011 cover crop, cereal rye, averaged 1,925 kg ha^{-1} of dry matter in organic plots and 2,400 kg ha^{-1} in integrated plots (Table 3-1). In 2011, soybean yields were statistically equivalent across treatments (one-way ANOVA, p-value = 0.426) averaging 3,148 kg ha^{-1} at 13% moisture content with less than 5% of the surface area of all plots with weedy species (Table 3-1).

Invertebrates

H. herbivagus and *B. rapidum* were excluded from analyses because too few individuals were captured. Significant interactions between year and treatment existed for 4 of the 9 taxa examined (*C. tricolor*, Gryllidae, Aranea, other carabids). Thus, data from 2010 and 2011 were analyzed separately for invertebrate captures and seed predation.

H. pensylvanicus activity-densities were not significantly effected by cropping system type in either 2010 or 2011 (Figures 3-1, 3-2). Beetles were active throughout the duration of sampling (July-September/October) in both years (Figure 3-3).

Cropping system had an effect on *P. melanarius* activity-densities in both of the study years (Figures 3-1, 3-2). Tukey's honest significance difference test revealed significantly ($p < 0.05$) more beetles in integrated plots versus no-till and organic plots in both 2010 and 2011. No difference in activity-density between organic and no-till plots was found in either of the study years (Figures 3-1, 3-2). This species' captures were low at the beginning of the study and became more frequent later in the year in both 2010 and 2011 (Figure 3-4).

Cropping system had an effect on *B. quad. oppositum* activity-density in 2010 but not in 2011 (Figures 3-1, 3-2). Tukey's honest significance difference test revealed significantly ($p < 0.05$) higher activity-densities in integrated and organic systems compared to no-till. Due to small sample size in 2011 a statistically significant difference could not be found, but no beetles were captured in no-till plots (Figures 3-1, 3-2). Beetles were most active in July in both study years (Figure 3-5).

C. tricolor activity-densities were significantly effected by cropping system type in 2010 but not in 2011 (Figures 3-1, 3-2). Tukey's honest significance difference test revealed significant ($p < 0.05$) differences in activity-density between all three cropping systems (integrated, no-till, and organic) in 2010. The highest activity density was observed in integrated plots, with organic plots having the second highest, and the fewest beetles captured in no-till plots (Figures 3-1, 3-2). In 2010, the majority of beetles were captured in July, while in 2011 few beetles were captured throughout the study (Figure 3-6).

The group composed of all unidentified carabid beetles was significantly effected by cropping system type in 2010 but not in 2011 (Figures 3-1, 3-2). Tukey's honest significance difference test revealed significantly ($p < 0.05$) higher activity-density in integrated and no-till systems compared to organic (Figures 3-1, 3-2). Activity-densities were lowest in the middle of August in both 2010 and 2011 (Figure 3-7).

Cropping system had an effect on Gryllidae activity-density in both of the study years (Figures 3-1, 3-2). Tukey's honest significance difference test analyses revealed significantly (p < 0.05) more captures in integrated plots versus no-till and organic plots in both 2010 and 2011. In 2011, organic plots had a higher activity-density than no-till (Figures 3-1, 3-2). Crickets were most abundant in July and early August with cropping system effects most pronounced at this time (Figure 3-8).

Aranea activity-density was effected by cropping system in both of the study years (Figures 3-1, 3-2). Tukey's honest significance difference test revealed significantly (p < 0.05) more captures in integrated plots versus no-till and organic plots in 2010. In 2011, integrated and organic systems had more captures than no-till (Figures 3-1, 3-2). As with crickets, spiders were most abundant in July and early August (Figure 3-9).

Cropping system had an effect on Opiliones activity-density in both of the study years (Figures 3-1, 3-2). Tukey's honest significance difference test revealed significant (p < 0.05) differences in activity-density between all three cropping systems (integrated, no-till, and organic) in 2010. The highest activity density was observed in organic plots, with no-till plots having the second highest, and the fewest individuals captured in integrated plots. In 2011, Opiliones activity-density was higher in organic plots compared to both no-till and integrated plots (Figures 3-1, 3-2). Integrated plots had relatively few captures in every trapping session (Figure 3-10).

In both 2010 and 2011, the most abundant granivores were *H. pensylvanicus* and Gryllidae. *H. pensylvanicus* accounted for 23.2% and 37.7% of all recorded potentially granivorous captures in 2010 and 2011, respectively. Gryllidae composed 15.8% and 21.8% of all granivorous captures in 2010 and 2011, respectively.

In 2010 and 2011, the six potentially granivorous taxa examined never had significantly lower activity-densities in integrated plots when compared to organic or no-till plots (Figures 3-1,

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3-2). In at least 1 year, 4 of these 6 taxa, *P. melanarius, B. quad. oppositum, C. tricolor*, and Gryllidae, had activity-densities significantly higher in integrated plots versus no-till plots. Likewise 4 of the 6 taxa, *P. melanarius, C. tricolor*, Gryllidae, and other carabids, had activity-densities significantly higher in integrated plots versus organic plots in at least 1 year of the study (Figures 3-1, 3-2). *H. pensylvanicus* was the only taxa examined that showed no response to cropping system in either year (Figures 3-1, 3-2). Members of class Arachnida, families Aranea and Opiliones, showed differing responses to the cropping system treatments. Over the course of the 2 year study, Opiliones activity-density was lowest in integrated plots while Aranea activity-density was highest in integrated plots (Figures 3-1, 3-2).

Activity-densities in organic plots were higher than those in no-till for 3 of the 6 potentially granivorous taxa, *B. quad. oppositum, C. tricolor*, and Gryllidae, and both of the Arachnid classes (Aranea and Opiliones) in at least one of the 2 study years. Activity-densities in no-till systems were only higher than organic plots for 1 taxa, other carabids, in 2010 (Figures 3-1, 3-2).

Seed Predation

No significant differences existed between the percentage of each seed species *(S. faberi, A. artemisiifolia,* and *A. theophrastii)* predated within a plot (GLM, p-value 0.430). As such, analyses were conducted on predation rate for all seeds combined rather than for separate weed seed species. In 2010, cropping system had an effect on seed predation with more seeds being devoured in integrated plots than organic plots (GLM, p-value = 0.023) with no significant difference in seed predation between no-till and the other cropping systems (Figures 3-11, 3-12). In 2010, weed seed predation ranged from 24-75% in the integrated system, 24-75% in the no-till system, and 8-50% in the organic system dependent upon the date of trapping. In 2011, no

difference in weed seed predation was found between cropping systems with predation rates ranging from 43-96% in the integrated system, 38-92% in the no-till system, and 46-81% in the organic system (Figures 3-12).

Gryllidae was the only taxa with a significant positive correlation to seed predation (p = 0.007). Though not significant, *H. pensylvanicus* and *P. melanarius* were marginally correlated with seed predation rates (p < 0.075). *Bembidion quadrimaculatum oppositum*, Aranea, Opiliones, and unidentified carabids other than the six granivorous species of interest had significant (p < 0.05) negative correlations with seed predation.

Discussion

The integrated weed management system employed in this study increased the activity-density of four of the five examined granivorous invertebrate taxa when compared to either no-till or organic systems. This integrated weed system was not detrimental to any granivorous invertebrate population or to weed seed predation. This supports my previous work (Law, chapter 2) showing that the application of a min-till method has little effect on invertebrate seed predator activity-density and invertebrate seed predation and is in agreement with research suggesting that cover crops through increased cover, humidity (Laub and Luna 1992, Clark et al. 1994), and food sources increase granivorous invertebrate densities (Manley 1996, Carmona and Landis 1999, Shearin et al. 2008, Ward et al. 2011).

However, seed predation was not significantly greater in integrated plots than conventional no-till plots in either year, despite a significantly greater number of invertebrate granivores. This is in contrast to previous work with cover crops by Davis and Liebman (2003) who found that a red clover crop underseeded in wheat led to a twofold increase in the number of giant foxtail seeds eaten by invertebrate granivores. In my study, the taxa with the largest

population increase in integrated plots was Gryllidae (crickets). The omnivorous field cricket, *Gryllus pennsylvanicus* Burmeister, composed the vast majority of these captures and is known to consume common ragweed, velvetleaf, redroot pigweed, large crabgrass, and giant foxtail seeds (Brust and House 1988, Carmona and Landis 1999). However, this species and *Allonemobius* sp. are also known to consume dead and alive plant matter, fruits, and both living and dead insects (Lundgren and Harwood 2012). In addition, these two taxa were shown to eat invertebrate prey when seeds were or were not present (Lundgren and Harwood 2012). Given the increase in both dead plant material and invertebrate prey in integrated plots, it seems that weed seed predation was not increased as crickets may have eaten more alternative food sources. It is reasonable to assume that omnivorous carabid species also ate more foods in addition to weed seeds.

Correlations between seed removal and taxon activity-density largely supported expected trends with activity-densities of the taxa known to be significant seed predators (Gryllidae, *H. pensylvanicus* and *P. melanarius)* being significantly correlated ($p < 0.05$) or marginally correlated ($p < 0.075$) with seed removal rates. Likewise, it was expected that activity-densities of Aranea, Opiliones, and unidentified carabids would exhibit significant negative correlations as was found. Aranea and Opiliones are predators of beetles (Drummond et al. 1990, Sunderland and Samu 2000) and given that the other carabids captured were not significant granivores, this group was largely composed of carnivorous beetles that would feed on and displace granivorous beetles. *B. quad. oppositum* activity-density was unexpectedly found to have a significant negative correlation with seed predation rates. Although this genus has been shown to be both mycophagous (Davies 1953, Sunderland 1975) and granivorous (Tooley and Brust 2002), a relatively large body of work shows this genus to be carnivorous (Coaker and Williams 1965, Baines et al. 1990, Sunderland 2002). It is likely that the diet of *B. quad. oppositum* is not regularly composed of a large portion of weed seeds and this species may displace granivorous invertebrates.

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The likely increase in alternative food sources was not detrimental to seed predation as predation levels were equal or greater in integrated plots compared to other plots. In addition, the increase in omnivorous invertebrates as a result of increased habitat complexity may enhance insect pest control while reducing predator interference by competition and intra-guild predation (Andow 1991, Landis et al. 2000). Likewise, the increase in Aranea (spiders) likely resulted in increased pest control as they are significant pest predators (Sunderland and Samu 2000). A decrease in Opiliones (harvestman) in integrated systems versus other systems may result in a decrease in pest control services as harvestmen are known to eat homopteran (Dixon 1989), coleopteran (Drummond et al. 1990) and lepidopteran (Ashby 1974) pests in agricultural fields. However, the majority of their diet has been shown to be composed of earthworms and plant material (Halaj and Cady 2000) and it is likely that their decrease in activity-density would not largely affect pest populations.

It should be noted that pitfall trapping was used to assess invertebrate abundance. It is possible that different proportions of the invertebrates present in different cropping systems were captured (see Melbourne 1999). Based on residue amounts, a higher proportion of individuals present in organic plots may have been captured as individuals could more quickly move through these plots. If this did occur, the difference in invertebrate abundance between systems would in fact be greater than the current data shows, with a higher number of invertebrates in integrated plots.

Our results are in agreement with a number of other studies that integrated weed management strategies can reduce herbicide application while effectively controlling weeds (Eadie et al. 1992, Buhler et al. 1995, Hanna et al. 2000, Blackshaw et al. 2008, Chikowo et al. 2009). Weed densities were equally low in all cropping systems with no significant differences in crop yield. Bates et al. (2012) found that the banded herbicide application in the row, coupled with select "surface tillage" operations (i.e., vertical coulter + rotary harrow + high residue

cultivation) between rows, used in this study provided a 70% reduction in the herbicide active ingredient applied compared to conventional herbicide-intensive management. Here I show that this tillage method in concert with the application of cover crops provides enhanced beneficial invertebrate populations.

My results indicate that the application of an integrated crop management system (i.e. surface tillage + cover crops) results in an increase in beneficial invertebrate activity-densities and does not decrease surface seed predation of three common weed species when compared to no-till and organic systems. The increased number of beneficial invertebrates did not result in increased weed seed predation rates but may have an effect on crop pests and merits further investigation.

Table 3-1: Mean cover crop biomass and grain yield by treatment.

	2009-2010				2010-2011			
	Vetch		Corn		Rye		Soybean	
	Yield (kg ha^{-1})							
Integrated	9,807	± 552	9,314	± 444	2,400	± 147	3,176	± 158
No-Till	n/a		10,060	± 389	n/a		3,312	± 132
Organic	9,780	± 620	9,212	± 1,045	1,925	± 565	2,955	± 132

No significant biomass or yield differences were observed between cropping systems (One-way ANOVA, $p < 0.05$). Mean ± standard error of the mean.
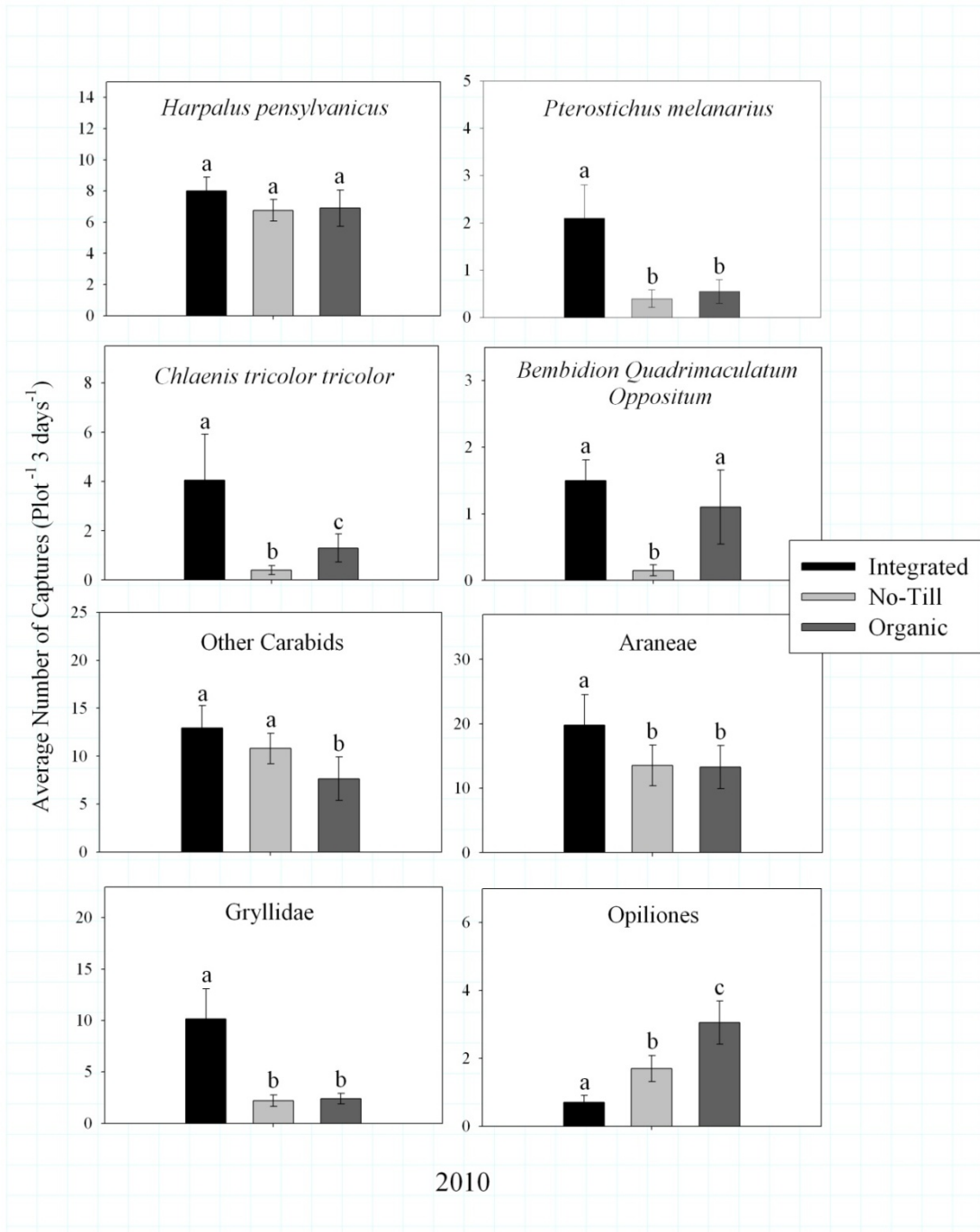
Figure **3-1**: Mean number of individuals captured per plot over 3 day trapping sessions throughout the study period (July-September) in 2010. Repeated measures generalized linear models followed by Tukey's HSD post hoc tests on the best model were used to test for treatment effects on the activity-densities of invertebrate taxa. Similar letters in a plot indicate no significant difference between treatments at $p < 0.05$. Vertical bars represent ± standard error of the mean.
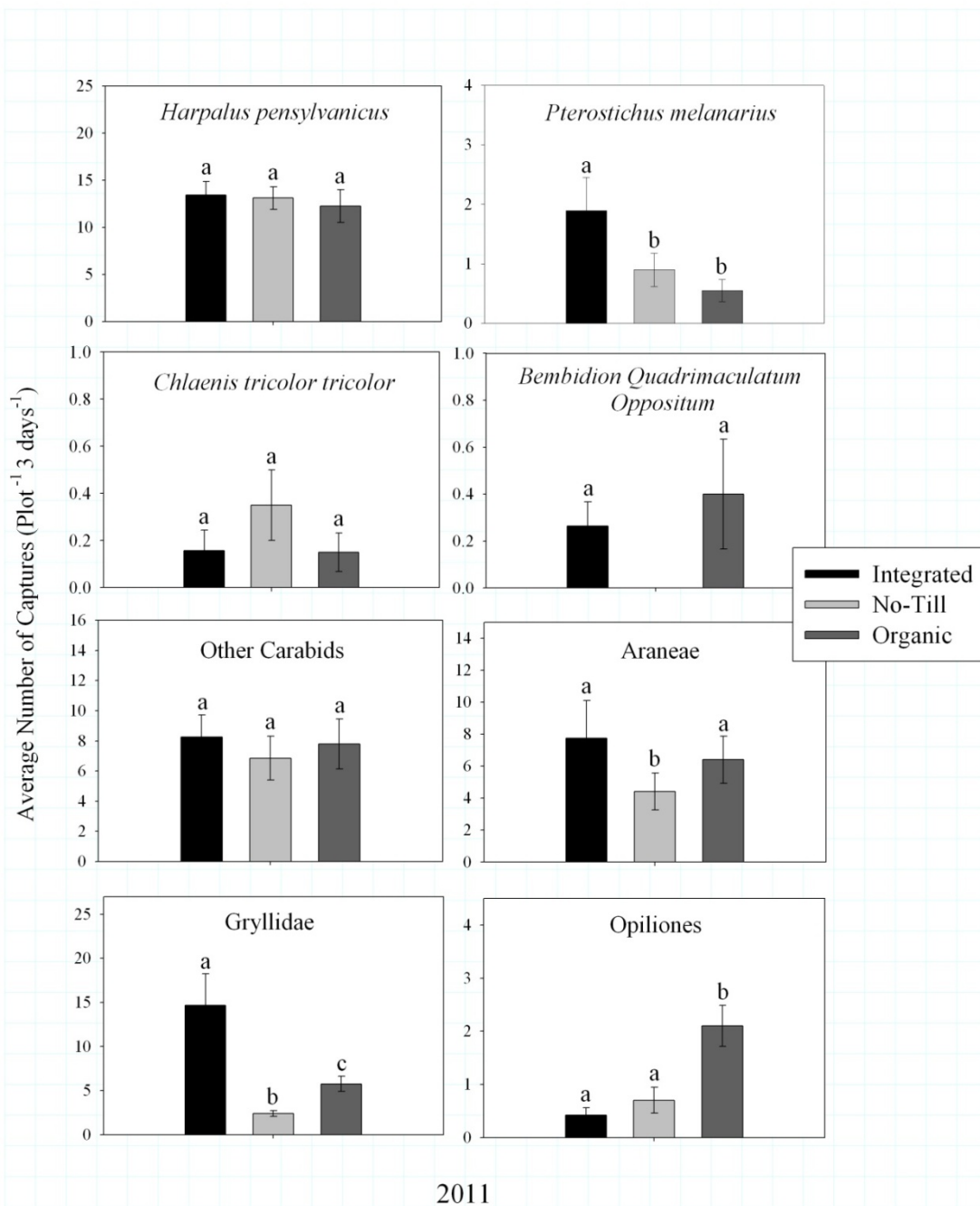
Figure **3-2**: Mean number of individuals captured per plot over 3 day trapping sessions throughout the study period (July-October) in 2011. Repeated measures generalized linear models followed by Tukey's HSD post hoc tests on the best model were used to test for treatment effects on the activity-densities of invertebrate taxa. Similar letters in a plot indicate no significant difference between treatments at $p < 0.05$. Vertical bars represent ± standard error of the mean.

Figure **3-3**: Mean number of *Harpalus pensylvanicus* individuals captured per plot over 3 day trapping sessions throughout the study period . Cropping system had no significant effect on activity-density in 2010 or 2011. Vertical bars represent ± standard error of the mean.

Pterostichus melanarius

Figure **3-4**: Mean number of *Pterostichus melanarius* individuals captured per plot over 3 day trapping sessions throughout the study period. Cropping system had an effect on activity-density in both of the study years. Tukey's honest significance difference test revealed significantly ($p < 0.05$) more beetles in integrated plots versus no-till and organic plots in both 2010 and 2011. No difference in activity-density between organic and no-till plots was found. Vertical bars represent ± standard error of the mean.
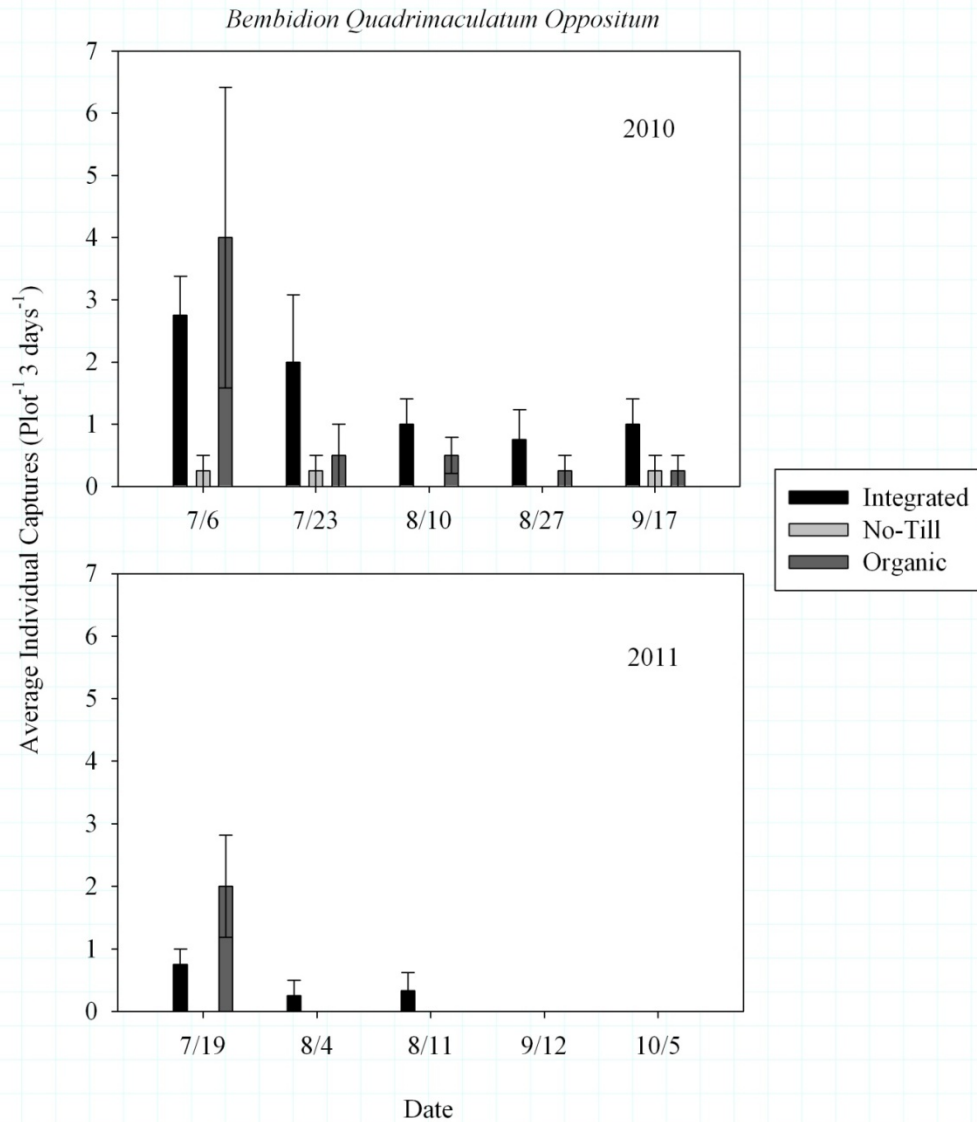
Figure **3-5**: Mean number of *Bembidon quadrimaculatum oppositum* individuals captured per plot over 3 day trapping sessions throughout the study period. Cropping system had an effect on activity-density in 2010 but not in 2011. Tukey's honest significance difference test revealed significantly (p < 0.05) higher activity-densities in integrated and organic systems compared to no-till in 2010. Vertical bars represent ± standard error of the mean.
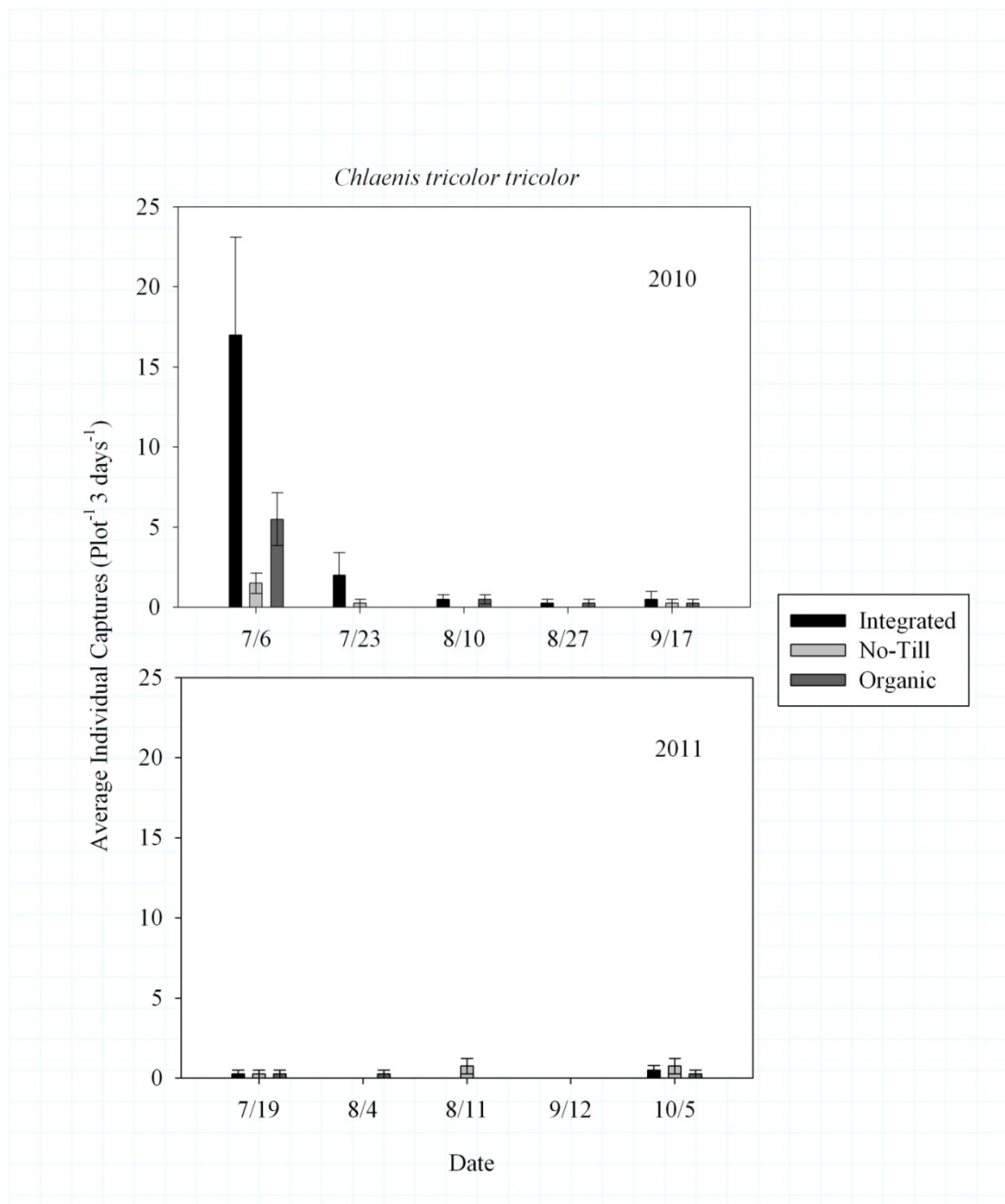
Figure **3-6**: Mean number of Chlaenis tricolor tricolor individuals captured per plot over 3 day trapping sessions throughout the study period. Activity-densities were significantly effected by cropping system type in 2010 but not in 2011. Tukey's honest significance difference test analyses revealed significant ($p < 0.05$) differences in activity-density between all three cropping systems (integrated, no-till, and organic) in 2010. The highest activity density was observed in integrated plots, with organic plots having the second highest, and the fewest beetles captured in no-till plots. Vertical bars represent ± standard error of the mean.
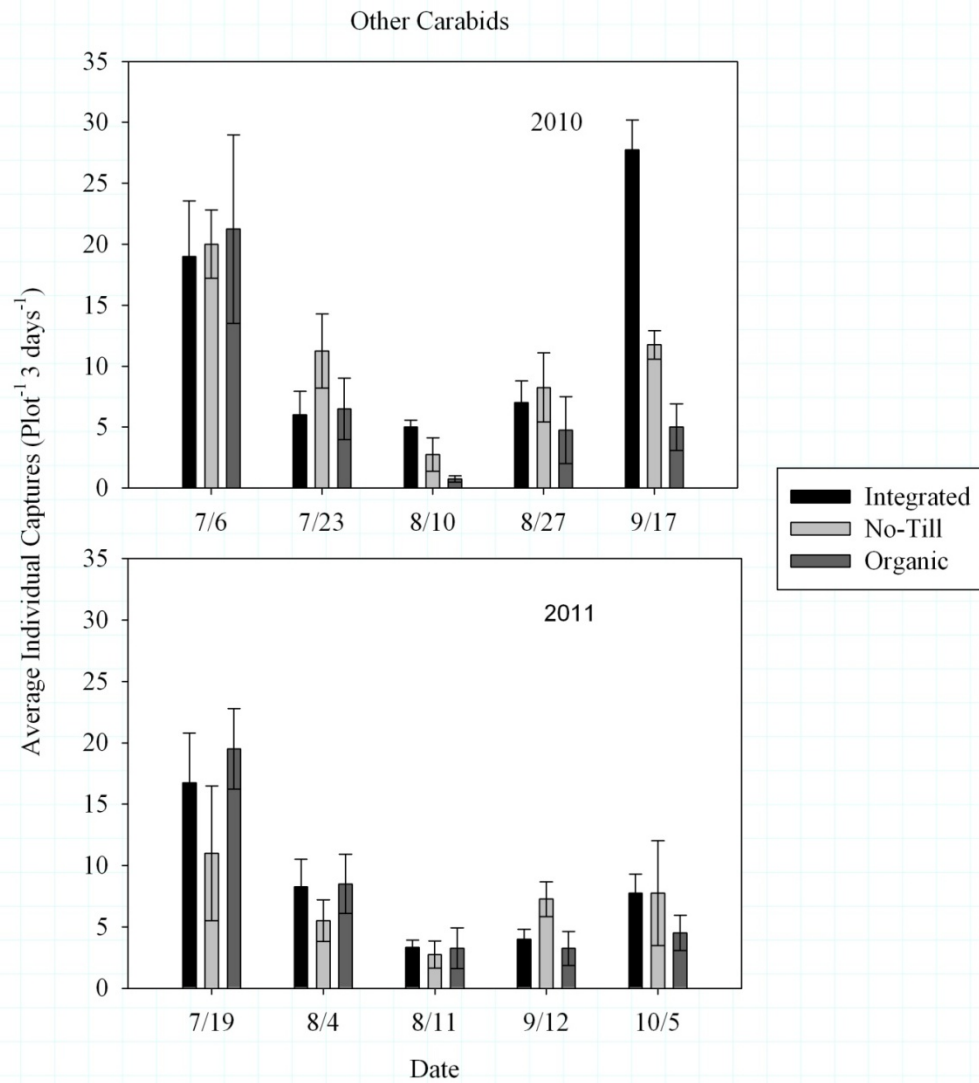
Figure **3-7**: Mean number of unidentified carabid individuals captured per plot over 3 day trapping sessions throughout the study period. The group was significantly effected by cropping system type in 2010 but not in 2011. Tukey's honest significance difference test revealed significantly ($p < 0.05$) higher activity-density in integrated and no-till systems compared to organic. Activity-densities were lowest in the middle of August in both 2010 and 2011. Vertical bars represent ± standard error of the mean.
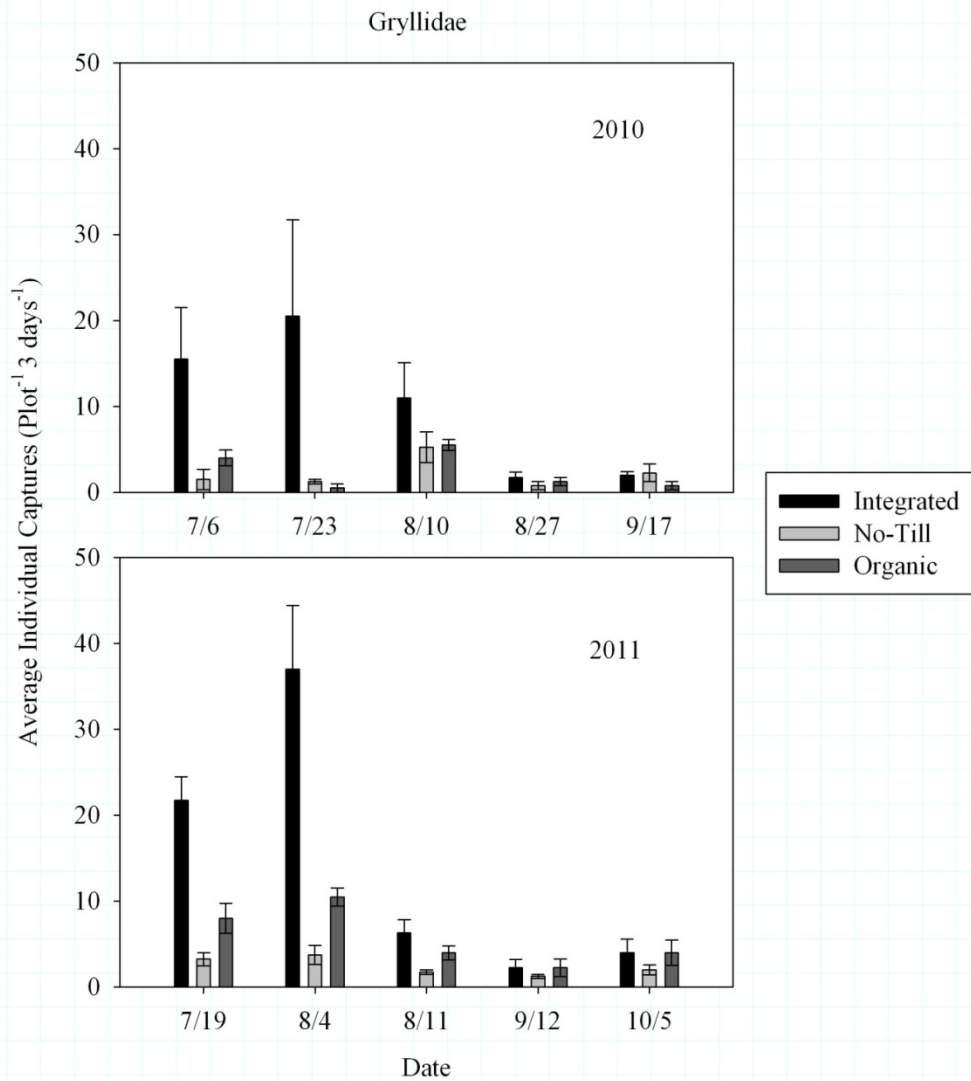
Figure **3-8**: Mean number of Gryllidae individuals captured per plot over 3 day trapping sessions throughout the study period. Cropping system had an effect on activity-density in both of the study years. Tukey's honest significance difference test revealed significantly ($p < 0.05$) more captures in integrated plots versus no-till and organic plots in both 2010 and 2011. In 2011, organic plots had a higher activity-density than no-till. Vertical bars represent $\pm$ standard error of the mean.
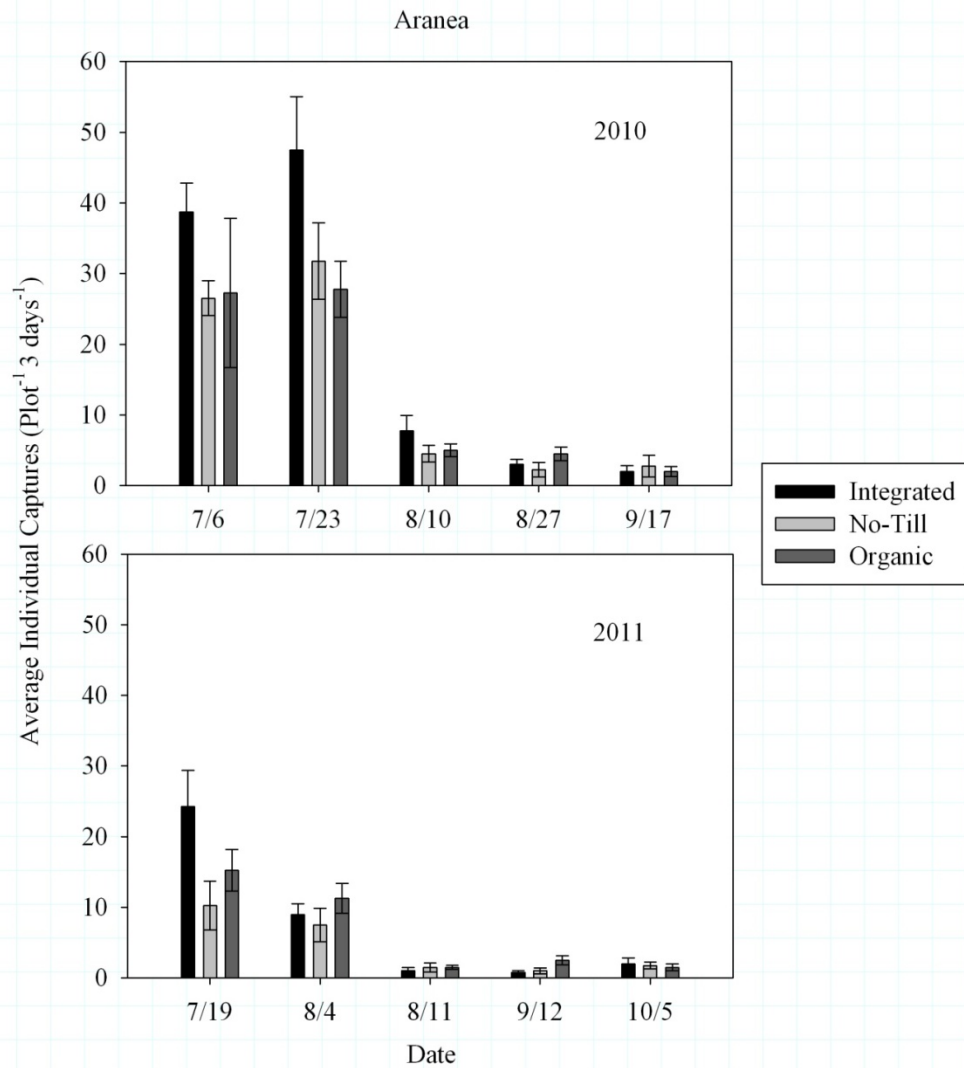
Figure **3-9**: Mean number of Aranea individuals captured per plot over 3 day trapping sessions throughout the study period. Activity-density was effected by cropping system in both of the study years. Tukey's honest significance difference test revealed significantly ($p < 0.05$) more captures in integrated plots versus no-till and organic plots in 2010. In 2011, integrated and organic systems had more captures than no-till. Vertical bars represent $\pm$ standard error of the mean.
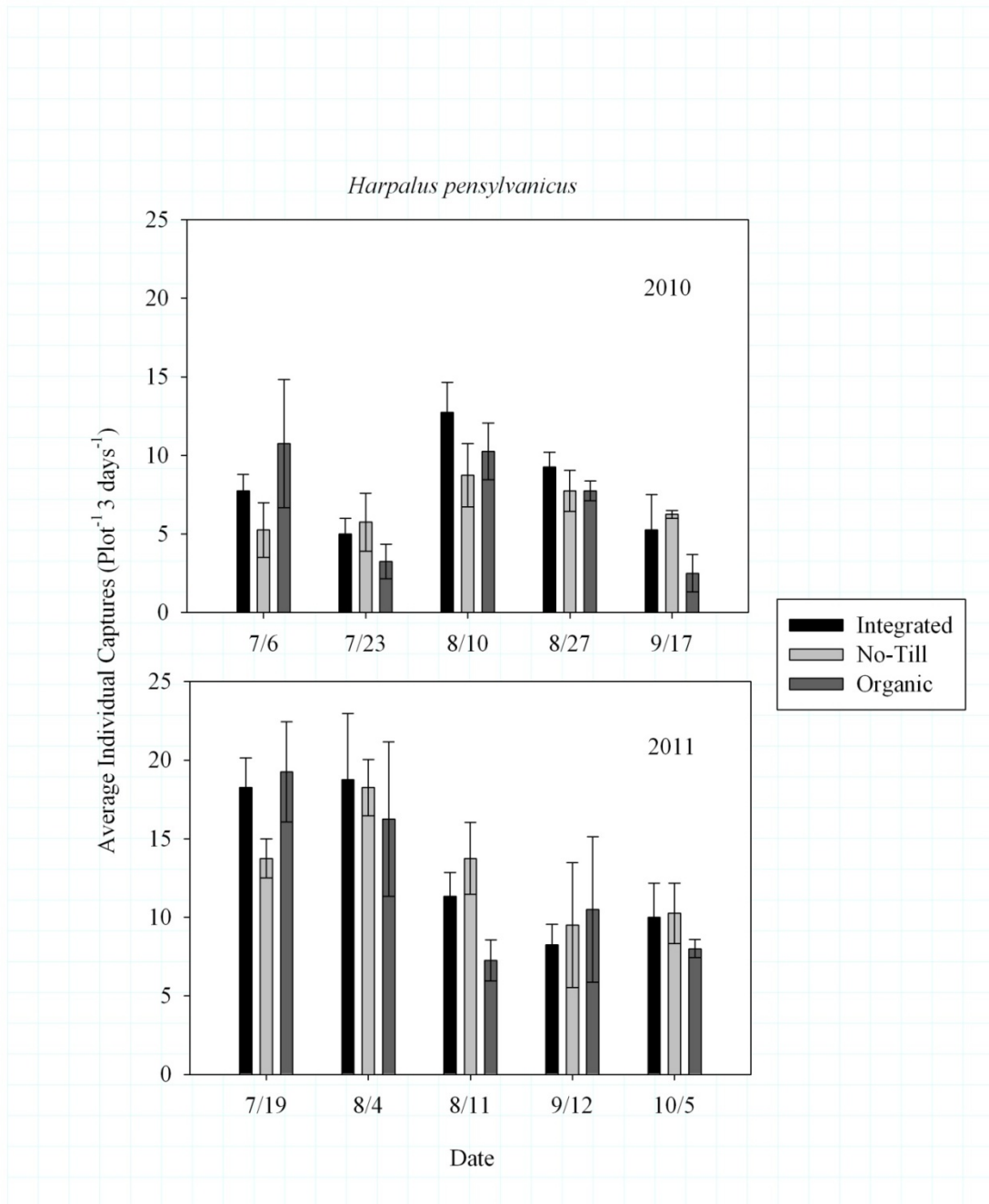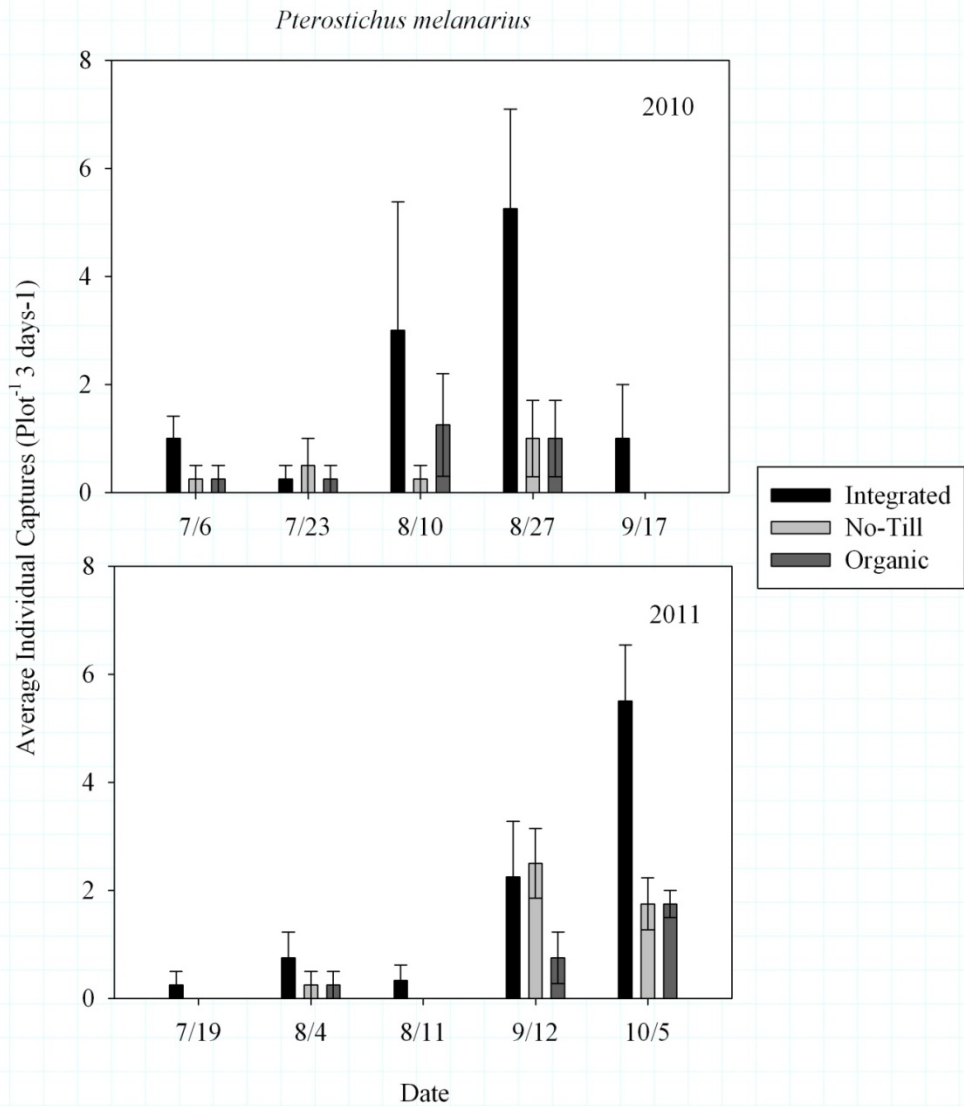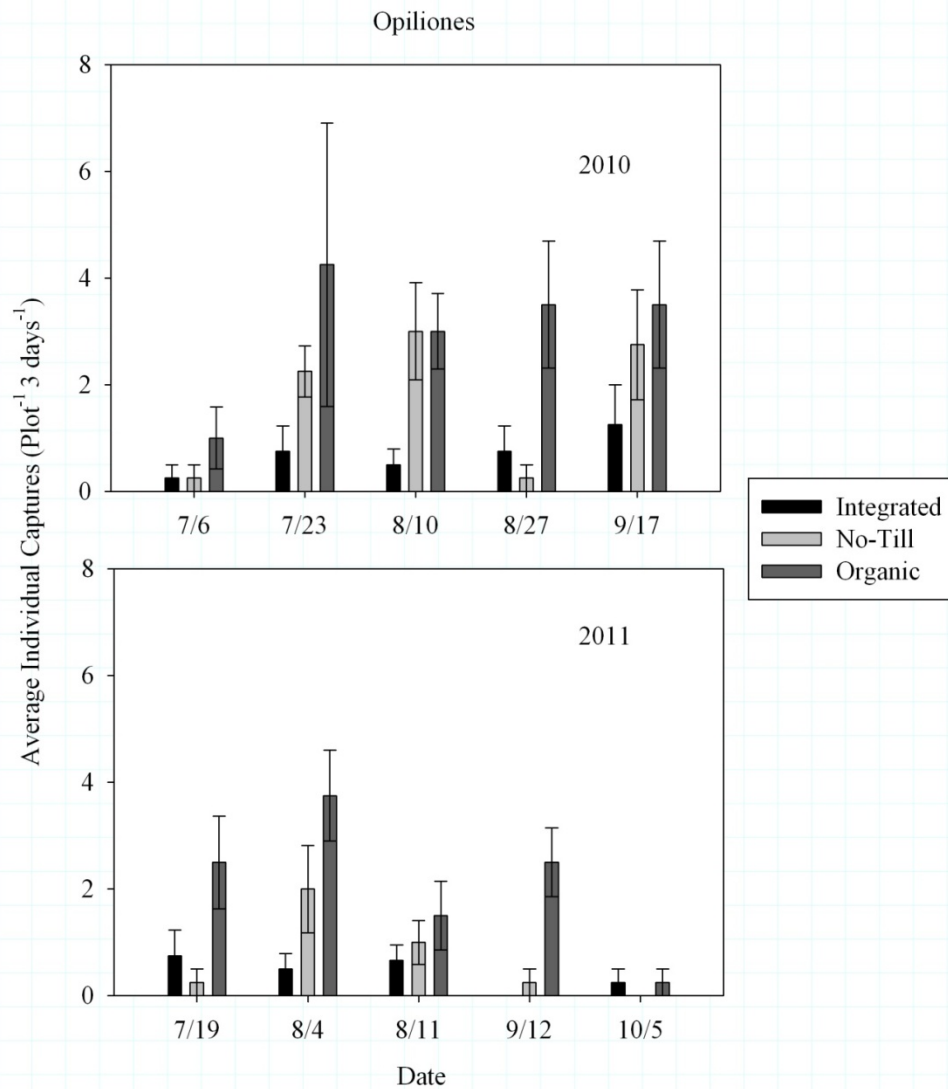
Figure **3-10**: Mean number of Opiliones individuals captured per plot over 3 day trapping sessions throughout the study period. Cropping system had an effect on activity-density in both 2010 and 2011 Tukey's honest significance difference test revealed significant ($p < 0.05$) differences in activity-density between all three cropping systems (integrated, no-till, and organic) in 2010. The highest activity density was observed in organic plots, with no-till plots having the second highest, and the fewest individuals captured in integrated plots. In 2011, Opiliones activity-density was higher in organic plots compared to both no-till and integrated plots. Vertical bars represent ± standard error of the mean.
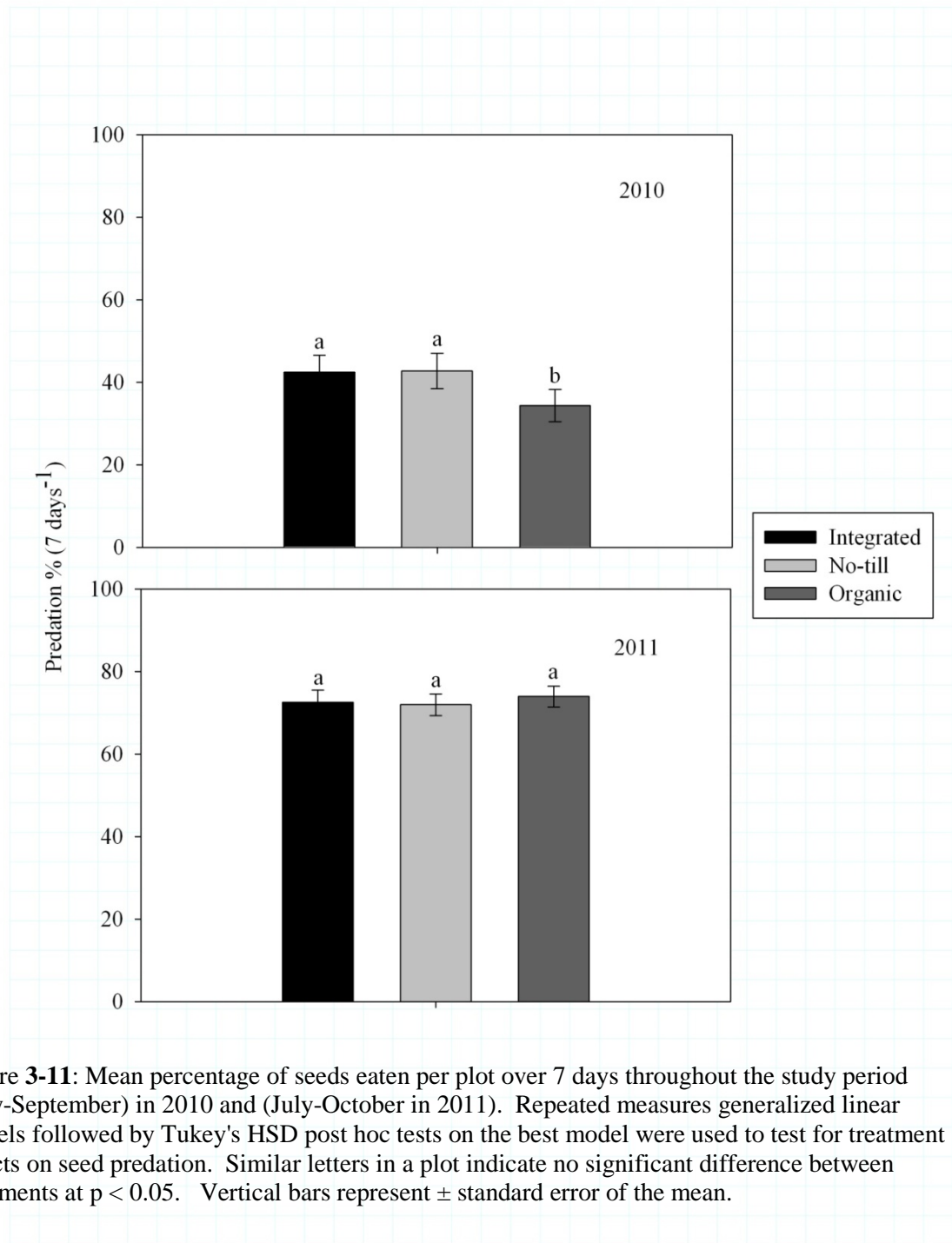
Figure **3-11**: Mean percentage of seeds eaten per plot over 7 days throughout the study period (July-September) in 2010 and (July-October in 2011). Repeated measures generalized linear models followed by Tukey's HSD post hoc tests on the best model were used to test for treatment effects on seed predation. Similar letters in a plot indicate no significant difference between treatments at $p < 0.05$. Vertical bars represent ± standard error of the mean.
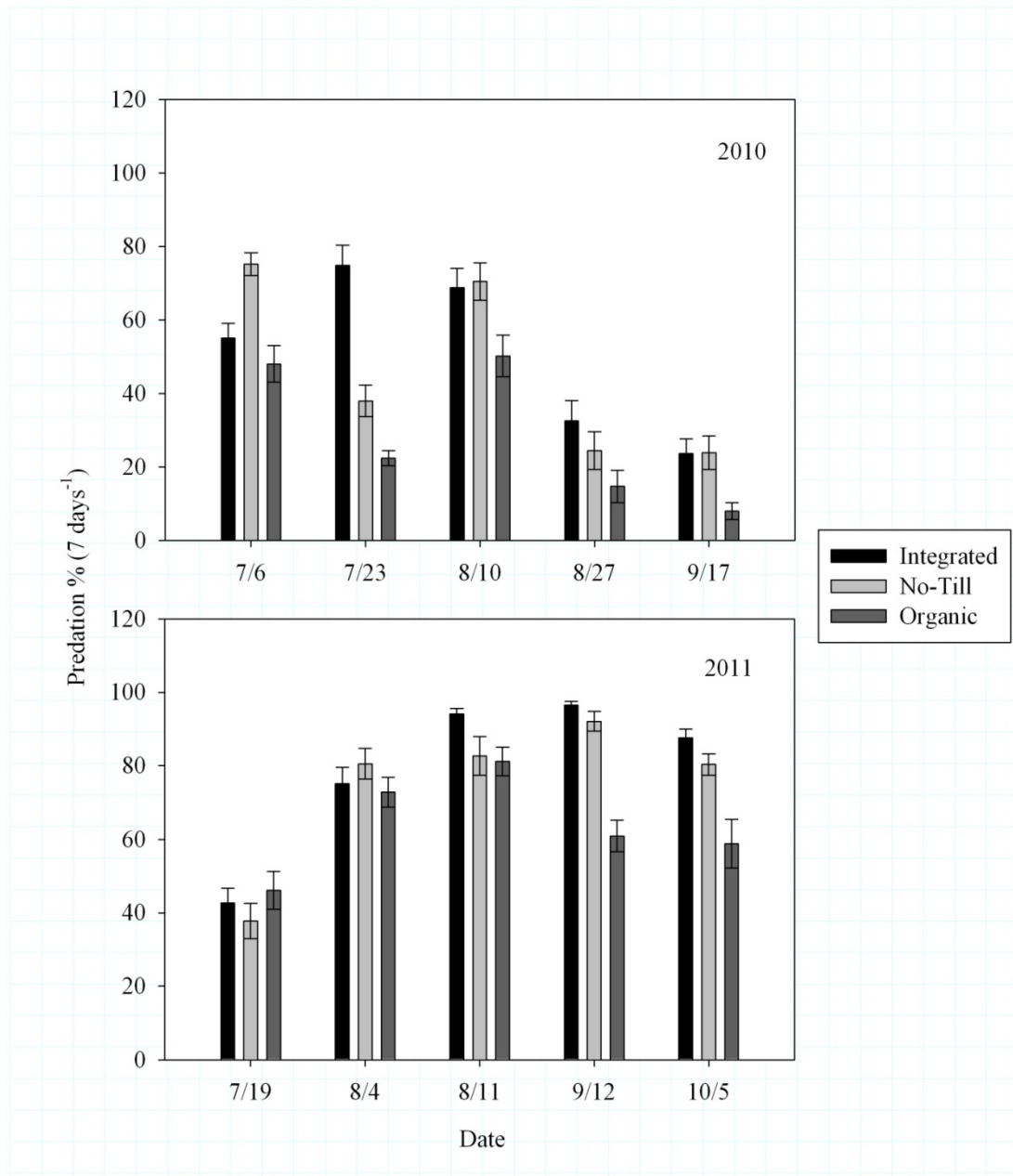
Figure **3-12**: Mean percentage of seeds eaten per plot over 7 days in each of the 5 trapping sessions in 2010 and 2011. Repeated measures generalized linear models followed by Tukey's HSD post hoc tests on the best model were used to test for treatment effects on seed predation. In 2010, significantly ($p < 0.05$) more seeds were eaten in integrated and no-till plots than organic, with no other differences observed. No significant differences in seed predation were observed in 2011. Vertical bars represent ± standard error of the mean.

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Chapter 4

Invertebrate Seed Selection and the Role of Imbibition

Introduction

It is well documented that many invertebrate seed predators selectively choose to eat certain seed species over others (see review in Lundgren, 2009). The degree of selectivity varies greatly and seed choice is thought to be influenced by a range of factors related to handling time and return investment including: seed size and morphology (Brown et al. 1975, Inouye et al. 1980, Hartke et al. 1998, Honek et al. 2007), seed density (Lundgren and Rosentrater 2007), nutritional quality (Janzen 1969, Inouye et al. 1980, Hendry et al. 1994), and phenology (Honek et al. 2005). Seed selection is a dynamic process that varies spatially and temporally and has the ability to alter weed community dynamics in agricultural fields.

Studies have found a variety of preferences (a disproportionally higher use of some groups of seeds compared to others) amongst carabids, with some research showing a preference for broadleaf species (Brust and House 1988, White et al. 2007), while others show a preference for grass species (Lund and Turpin 1977, Saska 2008). Brust and House (1988) documented a general preference for broadleaf weed species among carabids resulting in a significant shift in dominance of the community toward grass species over a three year period. The work of Brust (1994) has also showed that selective feeding by carabids reduces the above ground biomass of broadleaf weed species. Although no studies report total eradication of weed seeds by granivorous carabid beetles, it is quite apparent that weed dynamics are greatly altered through selective feeding and the resultant reduction in biomass. Species-specific preferences of carabids could influence the dominance patterns of weed species resulting in weed communities that are either more difficult or easier to control with available tools. It is imperative to understand

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carabid weed seed selection as this is a key ecological and economic interaction in agroecosystems.

One factor largely unaccounted for in seed preference studies is the role of imbibition. Of the numerous studies examining seed preference of carabids, only a select few have examined imbibed seeds (Cardina et al. 1996, Harrison et al. 2003). Both studies found imbibed seeds to be more accessible to carabid beetles. Cardina et al. (1996) found that the hard seed coat of velvetleaf was a deterrent to all invertebrate predators examined and only 2 predators would eat dry seed. Harrison et al. (2003) worked with imbibed smooth pigweed (*Amaranthus hybridus*), yellow foxtail (*Setaria lutescens*), and giant ragweed (*Ambrosia trifida*) after observing that dry ragweed seeds were not consumed by *Harpalus pensylvanicus*. It seems likely that imbibition is an important factor in determining seed palatability given that imbibed seeds have softer seed coats, are often metabolically active, and are frequently found in this condition in agricultural fields. My goal is to determine if seed preference by *H. pensylvanicus* is the same when seed is dry as opposed to imbibed. Optimal foraging theory predicts larger seed will be preferred over small seed in order to minimize handling time and maximize net energy intake (Schoener 1971). I hypothesize that the softer seed coat of imbibed seed will allow beetles access to larger seeds (i.e., ragweed (*Ambrosia artemisiifolia*) and velvetleaf (*Abutilon theophrastii*) and these imbibed weed seed species will be eaten in greater numbers than when dry.

The majority of studies examining carabid seed preference have been conducted in laboratories rather than the natural environment due to difficulties in obtaining seed selection data in the field. While providing information about potential feeding habits, these studies can do no more than hypothesize about what is occurring when beetles have access to the diversity of food sources available in the field. Laboratory studies eliminate or reduce factors that can affect food selection including intraspecific and interspecific competition, habitat complexity and heterogeneity, and altering food availability and foraging costs. Given the omnivorous feeding

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habits of most carabids (Lundgren 2009) and their potential role as weed and pest predators, it is important to understand their feeding habits in the natural environment

Field studies often rely on correlation analyses to gain understanding of food webs. Field observations, stomach content analyses, and natural carbon and nitrogen stable isotope ratios provide a window into the diet of invertebrate species but are limited because they do not identify the seed species devoured (stomach content analysis and carbon and nitrogen stable isotope ratios) or are too difficult to sample in order to obtain to provide sufficient information about the population (field observations). Due to methodological shortcomings it has been difficult to determine invertebrate food selection in the field. Here I use a novel application of stable isotope enrichment to mark seeds in the field in order to track their predation in a species-specific manner. This method allows me to determine if invertebrate species are consuming the weed seed species of interest by highly enriching seeds grown in the lab with a nitogen isotope, ^{15}N, and analyzing invertebrates with access to the seed for high amounts of ^{15}N in their tissues. I hypothesize that carabid beetle species, including *H. pensylvanicus* and *Pterostichus melanarius,* will eat both velvetleaf (*Abutilon theophrasti* Medic) and giant foxtail *(Setaria faberi* Herrm) seeds in a no-till field.

Materials and Methods

Cafeteria Choice Studies

Adult *H. pensylvanicus* were captured in a no-till field in August 2011 at the Russell E. Larson Agricultural Research Center in Rock Springs, PA located 16 km southwest of State College, Pennsylvania (Latitude: 40° 43' N Longitude: 77° 56' W). Individual beetles were placed in 30 plastic containers (33cm x 18cm x 11cm). Containers had a rubber base so that beetles could easily gain traction for movement. A paper towel provided cover and an insect

water pillow© (Zilla products, Franklin, WI) was used to provide water through the duration of the study. The experiment was carried out in a room with a temperature of 22 ±1 °C under an LD 14:10 h photoperiod. Each container had 0.189 mg of seven different seed species including velvetleaf (*A. theophrasti* Medic*),* common ragweed *(A. artemisiifolia* L.), redroot pigweed *(Amaranthus retroflexus* L.), common lambsquarters *(Chenopodium album* L.), barnyard grass *(Echinochloa crus-galli* L.), giant foxtail *(S. faberi* Herrm, and yellow foxtail (*Setaria lutescens* Weigel) mixed and placed within it. Enough seed was used to ensure that beetles would have access to an abundance of every seed species through the duration of the study. Fifteen of the containers had imbibed seed which had been placed on moistened filter paper for 24 hours prior to the study, while the other 15 repetitions remained dry. Imbibed seed was kept moist through the misting of water into the container daily throughout the experiment. After 120 hours seeds were removed, sorted, dried (in the case of the imbibed seeds) and weighed. A one-way ANOVA followed by Tukey's HSD post hoc analysis was used to determine if significant differences existed between the amount of imbibed and dry seed consumed for each weed seed species.

[15]N Tracer Study

In the spring of 2011, 15 individual plants of two species, *A. theophrasti* and *S. faberi*, were grown in a greenhouse at Penn State University, University Park, PA, USA. In order to track the predation of these plants' seeds in agricultural systems, plants were marked by a stable nitrogen isotope as outlined by Carlo et al. (2009). Upon flowering, flowers of both species were sprayed daily with ^{15}N-Ammonium Nitrate (98%+; Cambridge Isotope Labratories, Inc., Andover, MA, USA) at a concentration of 1 g L^{-1}. The solution also contained 5 mls of a polysorbate surfactant (Tween® 20) to improve adhesion and contact with plant surfaces. Once seeds were produced, they were harvested and stored at 4ºC.

In September 2011, 73g of collected *A. theophrasti* seeds were spread in 3 plots, 3m^2, each separated by 20m in a no-till field. Likewise, 55g of *S. faberi* seeds were placed in 3, 3m^2 plots, in a separate no-till field located approximately 1.5 km from field 1. Within and around each seed plot, 6 pitfall traps were placed flush with the soil surface. Pitfall traps consisted of an outer 950 ml plastic container (height 9.4 cm x diameter 11.4 cm) that contained a 140 ml plastic collection cup filled with propylene glycol (insect killing agent). Inverted funnels created from 2-liter bottles separated mammals from the propylene glycol and funneled the specimens into collection cups.

Invertebrate samples were collected daily from pitfall traps over 7 days. Concurrently, invertebrates not exposed to enriched seeds were captured via pitfall traps from a no-till corn field two kilometers away from the nearest enriched seeds. These specimens served as negative controls and were used as a means of comparison to determine if invertebrates captured during experimentation had eaten isotopically enriched seeds. In order to confirm that beetles eating enriched seed would have significantly higher $\delta^{15}N$ values, 15 *H. pensylvanicus* individuals, captured at the same location as the negative controls, were starved for 24 hours and subsequently exposed to isotopically enriched seed. Seven beetles were given access to enriched giant foxtail seeds and 8 beetles were given access to enriched velvetleaf seeds for 48 hours.

After capture, invertebrates were placed in a drying oven in preparation for ^{15}N analysis. All carabid individuals captured were analyzed for isotopic content but only selected individuals from other taxa were analyzed. Each individual sample was placed in a ball mill for 5 seconds to create a fine powder. One milligram of insect powder and 2 milligrams of seed powder for each collected sample were individually placed in 1.5 ml epitubes. Epitubes were mailed to EcoCore Analytical Services, Fort Collins, CO. EcoCore analyzed samples for $\delta^{15}N$ using a Delta V isotopic ratio mass spectrometer (IRMS), couple to a GC-isolink unit, with a Trace GC Ultra (Thermo Scientific). Students t-tests were conducted in R (R: A Language and Environment for

Statistical Computing 2011, R Development Core Team, Vienna, Austria) to compare δ^{15}N values of beetles exposed to isotopically enriched seeds and the negative control beetles. Students t-tests were also used to compare δ^{15}N values of enriched velvetleaf and giant foxtail seeds.

Results

Cafeteria Choice Studies

$\quad$ *H. pensylvanicus* ate significantly more imbibed than dry seed of all seed species (One-way ANOVA, p<0.001)(Figure 4-1). When dry, seeds of giant foxtail, barnyard grass, lambsquarter, and redroot pigweed were eaten in similar amounts with little predation of velvetleaf and common ragweed. However when imbibed, *H. pensylvanicus* ate most of the seed belonging to the two grass species, giant foxtail and barnyard grass, but also showed considerable predation of the other four species of weed seed (Figure 4-1).

^{15}N Tracer Study

$\quad$ The δ^{15}N values of *A. theophrasti* and *S. faberi* seeds from plants sprayed with ^{15}N-ammonium nitrate were on average more than 15-fold higher than seeds that were not sprayed, and had similar concentrations of ^{15}N to each other (Figure 4-2). Positive control beetles (those that ate seeds enriched with ^{15}N in the lab) served to create an expected profile of the ^{15}N values of wild beetles that ate enriched seeds. In the lab, beetles that ate enriched seed were significantly more enriched than negative control beetles (one-way t-test, p = 0.0025; data not shown).

$\quad$ It was expected that ^{15}N enrichment levels of invertebrates that ate enriched seed would be orders of magnitude higher than ^{15}N levels of naturally occurring individuals, and

determination of enriched seed predation could be made in the absence of negative controls. This was not the case and taxon specific negative controls were needed to determine if enriched seeds were eaten. Therefore, a determination if enriched seeds were eaten was able to be made for three invertebrate taxa (*H. pensylvanicus*, diplopods, and araneaeids) that had a sufficient number of negative controls collected in the field. An individual was considered enriched when its $\delta^{15}N$ was 2 standard deviations or greater than the mean of the negative control. For all other field collected invertebrates, it could not be determined if enriched seed predation had or had not occurred (Appendix).

Adult *H. pensylvanicus* and diplopods ate enriched weed seeds (Figure 4-3). Of the 31 individual *H. pensylvanicus* captured in plots with enriched giant foxtail, six (19.4%) had eaten enriched seed. In plots with enriched velvetleaf, 3 out of 31 *H. pensylvanicus* captured (10.3%) were enriched. One out of 7 analyzed diplopods ate enriched giant foxtail, with no obviously enriched individuals out of the 5 individuals from enriched velvetleaf plots. No spiders appeared enriched with ^{15}N above natural abundance values.

Discussion

Small and medium-sized seeds were eaten in greater amounts than large seeds by *H. pensylvanicus*, with little to no predation of large-seeded species when seeds were dry. However I found here that imbibition affects seed selection, and once seeds were imbibed, significantly more large seeds were eaten. Seed size is thought to be a major driver in seed selection by carabids. Honek et al. (2007) examined predation of 28 dicotyledonous seed species by 30 carabid beetle species and found that carabid beetle preferences were driven by taxonomic and size constraints. A body of research on *H. pensylvanicus* confirms my result showing that this species prefers small and medium-sized dry seeds. Ward (2008) found that common

lambsquarters and giant foxtail were preferred over velvetleaf. Likewise, Lundgren and

Rosentrator (2007) found that *H. pensylvanicus* has a preference for small seeds and Lund and

Turpin (1977) showed this species preferred medium-sized seeds. Only one study has illustrated

a preference by *H. pensylvanicus* for larger seeded species (White et al. 2007) and in fact, many

studies show, as ours did, that *H. pensylvanicus* will not eat any (or very little of) species such as

velvetleaf and common ragweed when dry. Ward (1998), Cardina et al. (1996), and Lundgren

and Rosentrator (2007) all illustrate that large-seeded species (either velvetleaf or ragweed) are

not preyed upon by *H. pensylvanicus*. However, Ward (1998) and Lundgren and Rosentrator

(2007) only examined dry weed seeds.

In one of the few studies to examine imbibed seed, Harrison et al. (2003) found that 62%

of *H. pensylvanicus* preferred small/medium seeded species with 17% preferring the larger

seeded species, giant ragweed. The work of Harrison et al. (2003) supports my result of altered

seed selection due to imbibition even though dry and imbibed seed selection was not directly

compared. Harrison et al. (2003) reported that imbibed seeds were used in this study because dry

giant ragweed was not eaten by *H. pensylvanicus*. Cardina et al. (1996) found that velvetleaf was

not eaten by *H. pensylvanicus* when dry or imbibed, which seems to contradict my results.

However, seeds in the Cardina et al. study were imbibed by placement in a moist paper towel for

8 hours as opposed to 24 hours in my study. It is possible that this hard-coated species was not

fully imbibed in the 8 hour time frame and the seed coat may have remained hard and resistant to

predation.

In addition to altering the proportion of seed species eaten, imbibition also increased the

amount of each seed species eaten in imbibed versus dry trials. This may be due to improved

palatability of seeds resulting from increased release of volatile organic compounds, including

carbon dioxide, alcohols, aldehydes, olefins, ketones, and volatile acids (Linton and Wright

1993). Moreover, the hard seed coat (testa) of seeds may be difficult to penetrate and imbibition

likely weakens this and provides easier access to the endosperm or cotyledons. Field research

gives merit to this idea showing that increased seed predation occurs in agricultural fields during

times of rain. Lundgren et al.(2006) found a tenfold increase in the number of weed seeds

(lambsquarter, velvetleaf, redroot pigweed, ivyleaf morning glory, crabgrass, giant ragweed, and

giant foxtail) removed from crop fields during a week with rain versus adjacent weeks with no

rain. Likewise, Cardina et al. (1996) related a season of unusually high precipitation with high

levels of velvetleaf seed predation.

Our field study with ^{15}N enriched seeds supported my laboratory work showing that *H.*

pensylvanicus individuals readily eat both giant foxtail and velvetleaf. This is in contrast to

previous work showing that velvetleaf either will not be eaten or is largely not preferred and

would not be eaten when other food options are available (Cardina et al. 1996, Lundgren and

Rosentrater 2007, Ward 2008). This study took place in a no-till field with no imposed

limitations on food availability. Furthermore, research was conducted in late September and

early October at the peak of seed rain for many weed species (Ward 2008, personal observation)

and thus beetles likely had many other food options available in the field. The field received

15.75mm of rain throughout the week of sampling and it is possible that the seed devoured was

imbibed.

Of the seven diplopods collected and analyzed from enriched giant foxtail plots, one

(14.3%) met my criteria as having eaten enriched seeds (i.e., having a δ^{15}N value 2 standard

deviations or greater than the mean of the negative control). However, given the δ^{15}N value of

this individual was not higher than values naturally observed in other invertebrate taxa and the

relatively small sample size of negative controls obtained, it is impossible to say with any

certainty whether or not this diplopod ate isotopically enriched seeds. As such, more research is

needed to determine if diplopods eat weed seeds in agricultural fields. These results, if

representative of diplopod populations, support the work of Koprdová et al. (2010) which showed

that *Cylindroiulus caeruleocinctus* (Diplopoda) would eat weed seeds in laboratory trials. In their study this species ate 31 out of 41 seeds in no-choice experiments and ate all 11 seed species that were presented in combination with dead leaves (a preferred food source). If granivorous, diplopods may be an important weed seed predator in agricultural fields as previous research has shown them to be abundant in no-till fields representing as much as 28.9% of all invertebrates (Cromar et al. 1999). The results of my study provide insufficient evidence to determine if seeds are a regular part of diplopod diet and their role in agroecosytems merits further investigation.

The results presented further support a body of work showing that *H. pensylvanicus* is a major seed predator in terms of its abundance and propensity to eat weed seeds. This study showed that through imbibition of seeds, *H. pensylvanicus* can eat significantly more seeds and a more diverse group of seeds than previously believed. Isotopic enrichment of seeds provided a unique way to track seed predation and showed that *H. pensylvanicus* will eat large seeded weed species in an agricultural field. In addition, this study provided some support that diplopods, one of the most abundant invertebrates in agricultural fields, may eat weed seeds in an agroecosystem.
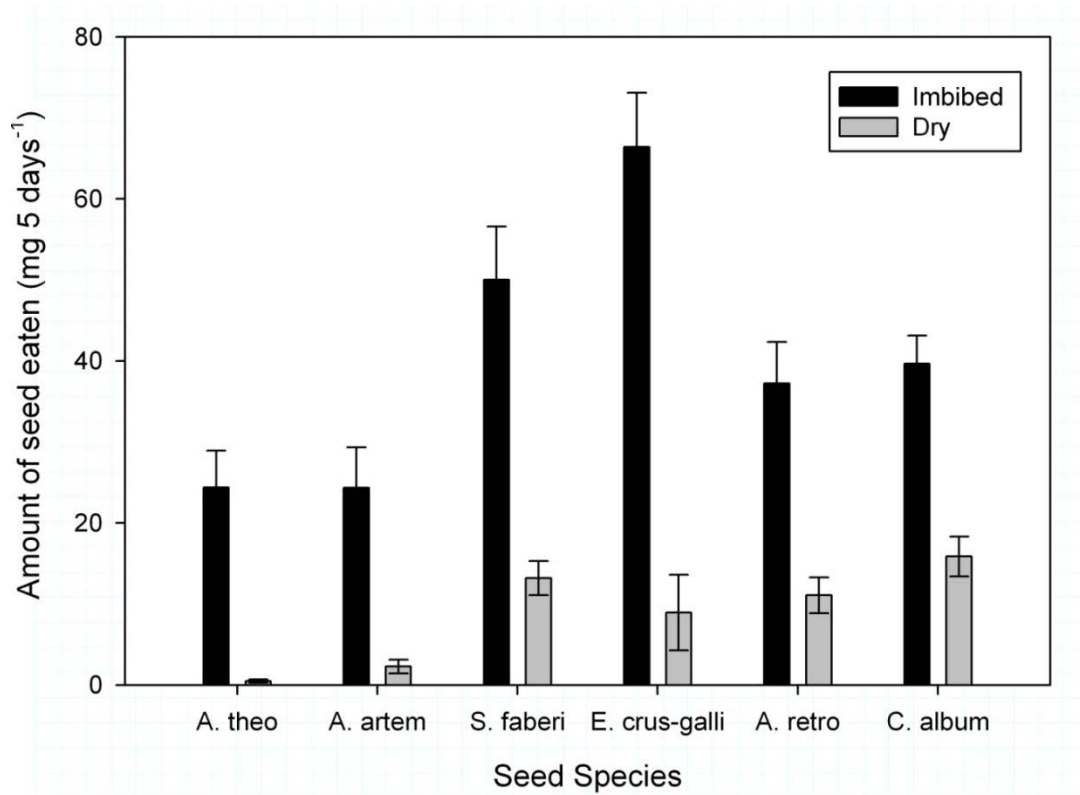
Figure **4-1**: Mean amount (mg) of dry and imbibed seeds consumed by *Harpalus pensylvanicus* in a cafeteria choice study over 5 days. Significantly more imbibed seeds were eaten than dry seeds of every species examined (One-way ANOVA; $F_{1, 168}$=160.045, P<0.001; Tukey's HSD post hoc tests, P <0.05). Vertical bars represent ± standard error of the mean.
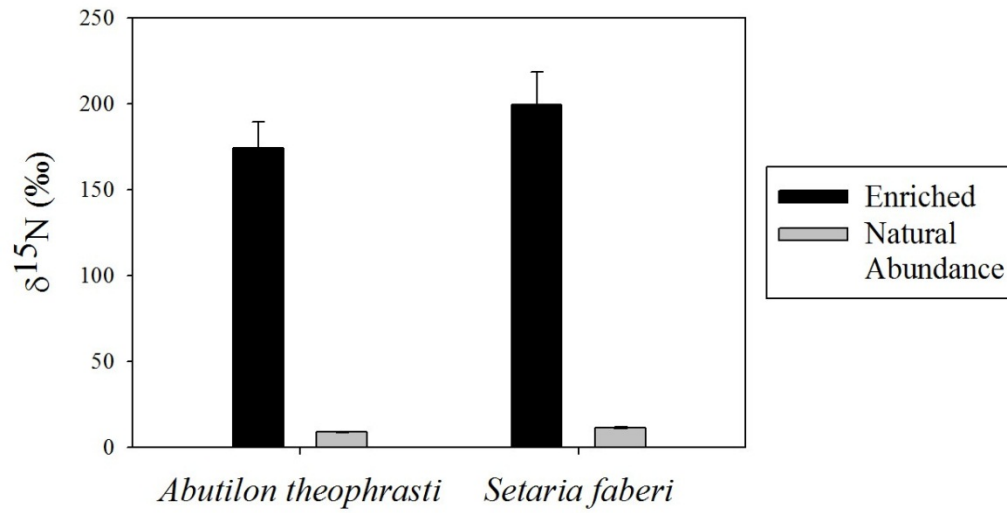
Figure **4-2**: Isotopic $\delta^{15}N$ values of naturally occurring seeds and those sprayed with ^{15}N ammonium nitrate (98%+). The two seed species from plants sprayed with ^{15}N-ammonium nitrate were similarly enriched (One-way ANOVA; $F_{1, 36}=1.286$, $P=0.264$) and significantly more enriched than the natural abundance samples (One-way ANOVA; $F_{1, 36}=208.093$, $P<0.001$). Vertical bars represent $\pm$ standard error of the mean.
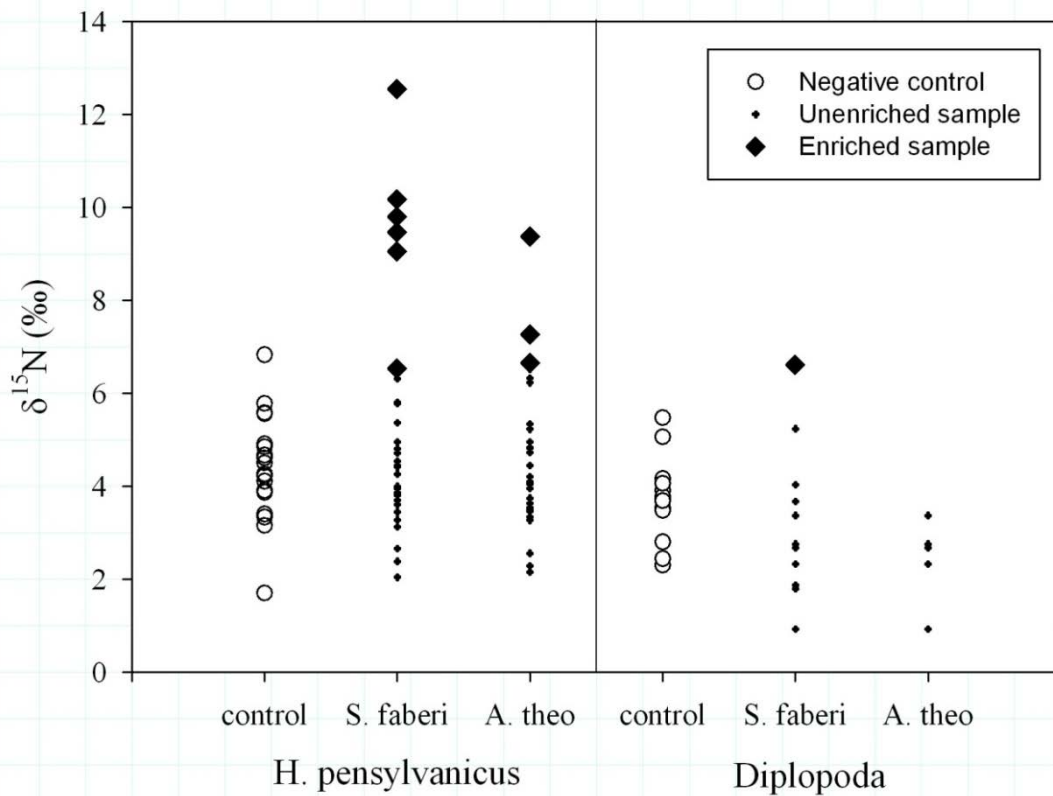
Figure **4-3**: Isotopic ratios of negative controls and field collected specimens of *Harpalus pensylvanicus* and diplopods exposed to either enriched *Abutilon theophrasti* or *Setaria faberi* seeds in no-till fields. Negative controls are field specimens collected that did not have exposure to isotopically enriched seeds. Unenriched samples are field collected specimens from a plot with isotopically enriched seeds that had a δ15N value within 2 standard deviations of the negative control. Enriched samples are field collected specimens from a plot with isotopically enriched seeds that had a $\delta^{15}N$ value 2 standard deviations or greater than the mean of the negative control.

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Chapter 5

VOC Release from Weed Seeds and Olfaction by *Harpalus pensylvanicus*

Introduction

Invertebrate seed predation has been shown to be an important component of weed seed loss in agricultural fields (Cromar et al. 1999), with carabid beetles (Coleoptera: Carabidae) shown to be important weed seed predators (Honek et al. 2003). As such, considerable attention has been paid to understanding the seed preferences of this taxon. Selective feeding on weed seeds has the potential to alter weed community composition and affect weed management decisions. One such example, Brust & House (1988) documented general preference for broadleaf weed species among carabids resulting in a significant shift in dominance toward grass species over a three year period. Seed selection is species specific and is known to be affected by seed size and morphology (Brown et al. 1975, Hartke et al. 1998, Honek et al. 2007), seed density (Lundgren & Rosentrater 2007), nutritional quality (Janzen 1969, Inouye et al. 1980, Hendry et al. 1994), and phenology (Honek et al. 2005).

The critical aspect, however, of how granivorous beetles locate food source is largely unknown. This information is important to help predict the degree of seed removal from the soil seed bank that can be expected by carabid beetles. For example, if beetles simply use visual, gustatory (i.e. taste), and tactile means to find food and determine its palatability, visually obscured seed would often be left untouched. Alternatively, if beetles sense weed seeds through olfaction and can frequently find visually obscured seed, they would be a more effective component of an integrated weed management strategy and could greatly reduce selected species in the weed seed bank. Although no direct evidence for the use of olfaction in finding seeds has been reported, germinating seeds have been shown to release a number of volatile organic

compounds, including carbon dioxide, alcohols, aldehydes, olefins, ketones, and volatile acids (Linton & Wright 1993), that predators may be able to detect. It is unclear, however, if dormant seeds release a similar spectrum of compounds.

There is evidence that carabids use olfaction to detect animal prey and habitat location. *Pterostichus melanarius* larvae and adults have been shown to sense a variety of volatile compounds including aphid alarm pheromone, live aphids, wheat extracts, live and dead slugs, and dipteran larvae (Kielty et al. 1996, Thomas et al. 2008). H*arpalus rufipes* sensed both aphid alarm pheromone and wheat extracts, and *Nebria brevicollis* responded to olfactory cues from collembolans (Kielty et al. 1996). *P. melanarius* has at least 4 types of receptors on their last antennal segment that may be responsible for detecting olfactory signals but the function of these receptors is unknown (Symondson & Williams 1997).

The indirect evidence available suggests that carabid beetles use olfactory capabilities in locating seeds. White et al. (2007) found that seed predation of redroot pigweed (*Amaranthus retroflexus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti)* seeds by *Harpalus pensylvanicus* did not differ between the soil surface and depths of 0.5 cm and 1 cm below the soil surface, suggesting that visually obscured seed can be located. Harrison et al. (2003) found that invertebrates, including carabids, preferentially choose to eat involucres that contain viable seed.

The objectives of this study are 1) to determine the volatile organic compounds released by seven common weed seeds in dry and imbibed states and 2) determine if *H. pensylvanicus,* a common weed seed predator known to prefer weed seeds as a food source (O'Rourke *et al.* 2006), would show a behavioral response to these compounds. I hypothesize that the weed seeds examined will release volatile organic compounds and that *H. pensylvanicus* will show a behavioral response to the seeds themselves and the constituent chemical compounds.

Materials and Methods

GC-MS Analysis of Volatile Organic Compounds

Two grams of ambient air dried seeds of seven common weed species, including velvetleaf (*Abutilon theophrasti* Medic*), common ragweed *(Ambrosia artemisiifolia* L.), redroot pigweed *(Amaranthus retroflexus* L.),common lambsquarters *(Chenopodium album* L.), barnyard grass *(Echinochloa crus-galli* L.), *giant foxtail (Setaria faberi* Herrm*),* and yellow foxtail (*Setaria lutescens* Weigel), collected at the Russel E. Larson Agricultural Research Center in Rock Springs, PA in August of 2010, were placed in 20ml GC headspace vials. For each weed species, there were two seed hydration states: 1) imbibed at 100% RH and 2) ambient dry. The imbibed state was achieved with the addition of 2mls of water to the vial, using a cotton plug suspended over the water to separate the seeds from the water. This allowed for the seeds to become slowly imbibed over time. The ambient dry state was achieved by sealing the cotton plug + seeds in the vial without the addition of any water. Vials were sealed with crimp-type headspace septa and metal seals. Three replicates for each weed species by seed hydration state were established. The vials were immediately transferred to a Thermo Scientific XYZ Headspace Incubator held at 30°C that worked in conjunction with Thermo Scientific® Trace Ultra® GC with a PTV® inlet, DSQ® MS and a TriPlus®autosampler (Thermo Fisher Scientific, West Palm Beach, FL). The sealed vials were sampled every three days over the course of nine days to determine the volatile organic compounds released by the seeds. The GC column was a 30m by 0.25mm ID Rxi®-5ms 30 (Restek, Bellefonte, PA) with helium as the carrier gas (1.5 ml/min). The syringe temperature was set to 70°C with an inlet temperature of 175°C. A 1ml sample was injected in the splitless mode. The GC oven was initially held at 35°C for 30 seconds, and then brought to a temperature of 140°C at a rate of 20°C per minute. Mass spectra were collected at the rate of 6 scans/second over the mass range (*m/z*) 50−650. The column was then held at 250°C for 30 seconds after the

run in order to clean it. Xcalibur® 1.4 (Thermo Fisher Scientific, West Palm Beach, FL) was used as the primary software interface with the GC-MS and for data processing.

Carbon Dioxide and Ethylene Release

To quantify amounts of carbon dioxide and ethylene released from the seeds of the seven weed species, a separate experiment using the identical species – seed hydration configuration outlined for the GC-MS experiment was used. The vials were incubated at $22 \pm 1°C$, and sampled on days 0, 3, 6, and 9. Ethylene content was determined by taking a 1ml sample from the headspace above the sealed seed container and analyzing it with a gas chromatograph (Hewlett-Packard 6890, Palo Alto, CA, USA) fitted with a flame ionization detector and an activated alumina column. Carbon dioxide content was determined by taking 1ml samples from the headspace above the seeds and analyzing it with a CO_2 /H_2O analyzer (Li-Cor LI-7000, Lincoln, NE, USA). The CO_2/H_2O analyzer was calibrated with known CO_2 concentrations on each day of sampling before analysis.

Olfaction Experiments

Adult *H. pensylvanicus* beetles (Coleoptera: Carabidae) were collected from pitfall traps located at the at the Russell E. Larson Agricultural Research Center in Rock Springs, PA, 16 km southwest of State College, PA (Latitude: 40° 43' N Longitude: 77° 56' W) in August and September of 2011. Beetles were kept in terrariums of moist soil in the laboratory at $22 \pm 1°C$ and were fed kitten food containing a minimum of 40% protein. *H. pensylvanicus* was chosen for this study because this species is a dominant weed seed predator (Westerman et al. 2008) found throughout North America and has been shown to be the most abundant carabid in the study area (Leslie et al. 2009). All olfaction bioassays were conducted after dusk and under red light (630

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nm LED array, 0.6 umol $m^{-2} s^{-1}$) to ensure the beetles' circadian rhythm and behavior was not disrupted.

Before examining olfaction, beetles were assessed to determine if they would respond to weed seeds when given full access to the seeds with all parts of their sensory system. Arena trials were conducted in which beetles were placed in a circular 20.5cm diameter dish and had complete access to seeds. Three seed species (common ragweed, velvetleaf, and giant foxtail), dry and imbibed, were individually assessed. Seeds to be imbibed were placed on moistened filter paper for 24 hours before arena trials. An individual beetle was placed in the arena with a 2g seed sample and the time spent foraging in the half of the arena nearest the seeds out of 10 minutes was measured. Beetles were considered interested in the seed if the majority of time >5 minutes was spent foraging in and around the seeds. Fifteen replicates were completed for each dry and imbibed seed species.

Olfactometer bioassays were conducted to determine if *H. pensylvanicus* individuals respond to volatile compounds being released from seeds, from the weed species used in the arena studies, when dry and in an imbibed state. Twenty-four hours prior to trials beetles were starved and seeds to be imbibed were placed on moistened filter paper. Bioassays were conducted in a 40-mm diameter × 36-cm long glass, Y-tube olfactometer that had a 50° inside angle. Purified air was supplied from a gas cylinder and was humidified via bubbling through a beaker containing distilled, deionized water. The purified air was split into two Erlenmeyer flasks; one flask served as a control (was empty) and the other chamber held the test material (i.e., dry or imbibed seeds). From each holding chamber, the air passed into the respective arms of the Y-tube, and then through a series of screens, to limit beetles access to the air source, before entering the main tube of the olfactometer. All airflow was directed with glass connections and Teflon® tubing due to its chemical resistance, stability, and anti-adhesive properties. Airflow through the system was maintained at 100 ml/min by two inline flowmeters. A smoke test

demonstrated laminar airflow in both arms and throughout the olfactometer. Individual beetles were introduced to the base of the y-tube and given 10 min to respond to the treatment. A choice for the left or right arm of the olfactometer was noted when the beetle went 1 cm past the Y junction and remained there for at least 15 seconds. Trials were conducted until 20 individuals made a choice for an arm of the olfactometer. The olfactometer was cleaned between each trial with a pH neutral liquid detergent (extran MA02), and rinsed with distilled water, hexane, and acetone to eliminate any chemical cues that may have been released by the beetles. Y-tube orientation was randomly altered and the arm of y-tube receiving volatile compounds was switched before each trial. Similar methodologies have been employed in determining olfactory responses of other coleopteran species (Heil 2004, Lacey et al. 2004, Stenberg & Ericson 2007).

The constituent chemicals of the volatile release from the weed seeds determined through gas chromatography (ethylene) and the CO_2 /H_2O analyzer (carbon dioxide) were used in beetle bioassays. No other organic volatile compounds were detected with the GC-MS. The assay set-up was similar to the above-mentioned trials. However humidified, purified air was provided to one arm of the olfactometer via a gas cylinder while the other arm received ethylene or carbon dioxide via another gas cylinder. Airflow was maintained at 100 ml/min and trials were conducted until 20 individuals choose an arm of the olfactometer for each chemical. Significant differences in the proportion of *H. pensylvanicus* choosing a particular olfactory source were tested using a two-sided binomial test (R: A Language and Environment for Statistical Computing 2011, R Development Core Team, Vienna, Austria).

Results

Analysis of Volatile Organic Compounds

There was no discernible evidence from the GC-MS analysis of organic volatile compounds from the dry or moist weed species seeds tested in this study (data not shown). However, analysis of the headspace with the CO_2 /H_2O analyzer indicated release of CO_2 from both the dry and moist seeds of all species examined (Table 5-1). In dry seed, carbon dioxide release from seeds was found for each seed species in at least one of the three time periods. The largest amount of CO_2 release for each seed species ranged from 5 mg l^{-1} g_{seed}^{-1} h^{-1} to 141 mg l^{-1} g_{seed}^{-1} h^{-1} dependent upon seed species. In moist seeds, maximum carbon dioxide release ranged from 6,567 mg l^{-1} g_{seed}^{-1} h^{-1} to 17,286 mg l^{-1} g_{seed}^{-1} h^{-1} dependent upon seed species. Carbon dioxide concentrations in the head space tended to increase with the 6 and 9 day incubation periods in the imbibed seeds, but not in the dry seed.

Analysis of the headspace with gas chromatography, specifically analyzing the samples for ethylene, showed the release of ethylene from both the dry and moist seeds (Table 5-2). Maximum ethylene release per seed species ranged from 2 µg l^{-1} g_{seed}^{-1} h^{-1} to 10 µg l^{-1} g_{seed}^{-1} h^{-1} in dry seed and from 11 µg l^{-1} g_{seed}^{-1} h^{-1} to 25 µg l^{-1} g_{seed}^{-1} h^{-1} in moist seed. The largest ethylene release for each moist seed species was found between days 6 and 9 but this trend was not true of dry seed.

Arena and Olfactometer Choice Studies

Arena bioassays showed that the majority of *H. pensylvanicus* beetles (86.7%-100%) were attracted to all 3 species of seeds, when given complete access to them, with no significant difference between the dry and imbibed seeds. In olfactometer bioassays, beetles showed a significant response to imbibed *A. theophrasti* and imbibed *S. faberi* seeds selecting them in 80%

and 75% of trials (binomial, $p < 0.05$), respectively, but no response to imbibed *A. artemisiifolia* (binomial, $p > 0.05$)(Table 5-3). Beetles did not show a significant response to any of the 3 seed species when dry (binomial, $p > 0.05$). Ethylene at 33 ppb and carbon dioxide at 800ppm did not elicit a significant response by beetles (binomial, $p > 0.05$). However, 11,400 ppm carbon dioxide showed a significant negative response by *H. pensylvanicus* with 75% of beetles moving to the arm of the olfactometer with purified air (binomial, $p < 0.05$)(Table 5-3).

Discussion

Two volatile organic compounds were found to be released by seeds before germination, carbon dioxide and ethylene. An increase in seed-born ethylene is associated with seed ripening and germination (Mattoo & Suttle 1991) and thus is a plausible cue for seed predators to locate seeds. Both chemicals have been shown to be detectable by invertebrates. The phytophagous corn earworm, *Helicoverpa zea*, produces sex pheromones as a function of ethylene concentration in its immediate microclimate (Raina et al. 1992) and more related to this study, *Phloeotribus scarabaeoides* (Coleoptera) has been shown to be attracted to ethylene (Campos & Peña 1995). Carbon dioxide is an olfactory stimulus for many invertebrates, including members of Lepidoptera, Diptera, Hymenoptera, Isoptera, Chilopoda, and Ixodidaesee (Stange & Stowe 1999).

Based on the Y-tube bioassays, *H. pensylvanicus* adults have the ability to detect imbibed seeds through olfaction when carbon dioxide and ethylene release are highest, but not when seeds are dry and carbon dioxide and ethylene release is relatively low. Although not shown in invertebrates, previous research has illustrated a similar pattern in vertebrates. Yellow pine chipmunks (*Tamias amoenus*) and deer mice (*Peromyscus maniculatus*), among other rodents, were shown to find significantly more seeds in moist rather than dry conditions (Vander Wall

1998). Although beetles in my study responded to imbibed seeds of two of three species examined, there was no evidence that carbon dioxide or ethylene were seed predator attractants. The only response observed to these chemicals was a negative response by *H. pensylvanicus* to high levels (1.14%) of carbon dioxide, demonstrating their ability to detect some concentrations of carbon dioxide. This concentration is higher than amounts released by seeds in my study and is more on the order of CO_2 values produced by mammal respiration (4-5%). It is possible that beetles avoid high CO_2 areas in an attempt to avoid predators as has been shown with other insects (Suh *et al.* 2004). An attraction response by *H. pensylvanicus* to CO_2 or C_2H_4 may require different concentrations or combinations of these chemicals. Indeed, Campos and Pena (1995) found that a narrow range of ethylene concentrations elicited attraction responses in the olive bark beetle. Although I found evidence of an olfactory response, the chemical nature of the olfactory cue is still unknown.

Seed hydration affects seed selection, and it is therefore likely that seed predation studies using dry seeds will not have results representative of the seed selection occurring in a natural and often imbibed environment. Future studies should include imbibed seeds to determine selective pressures under different environmental conditions. My study suggests that the moisture level of the environment has implications for seed detection and thus the amount of weed seed entering the seed bank. In moist years, beetles are capable of finding more obscured seeds while in dry years, visually obscured seeds are less likely to be eaten and more likely to enter the seed bank. One of the seeds tested, *A. artemisiifolia,* was not significantly detected by beetles when dry or imbibed. *A. artemisiifolia* has a hard involucre that covers the seed and may reduce the amount of water reaching the seed. Seeds in this study were placed on moistened filter paper for 24 hours and that may not have been a sufficient time to penetrate the involucre of this species. This suggests that infrequent rain events, which often occur in summer months, may lead to more

selection pressure of soft seed coated weedy species and allow hard coated species, such as *A.*

artemisiifolia, to become more abundant.

Table **5-1**: The amount of carbon dioxide released (ppm) by 1 gram of seeds in 1 hour. Means ± standard error of the mean.

Species	Type	Days 0-3			Days 3-6			Days 6-9		
Setaria faberi	D	12.71	±	4.95	10.94	±	14.05		nd	
	M	232.66	±	11.34	6867.11	±	1055.34	10321.21	±	133.98
Amaranthus retroflexus	D	18.41	±	10.31	50.91	±	47.47		nd	
	M	904.56	±	449.38	10073.22	±	2018.89	8300.40	±	1858.40
Chenopodium album	D	72.59	±	22.46	140.83	±	128.09		nd	
	M	1432.95	±	164.10	5640.84	±	1470.86	12520.90	±	1350.09
Abutilon theophrasti	D	16.85	±	5.29		nd			nd	
	M	536.10	±	83.93	6422.38	±	1265.93	10323.47	±	1596.20
Ambrosia artemisiifolia	D	12.61	±	5.65		nd		11.63	±	11.15
	M	155.33	±	72.28	8502.96	±	1326.05	7753.64	±	1089.68
Setarialu lutescens	D	5.79	±	2.23	0.11	±	3.82		nd	
	M	38.20	±	7.20	464.11	±	441.12	17285.93	±	2118.54
Echinochloa crus-galli	D	5.25	±	3.89	4.29	±	1.17	0.06	±	1.66
	M	159.19	±	21.72	4319.35	±	431.14	6566.91	±	584.65

D: Seeds were kept dry under ambient humidity. M: Seeds were kept moist under high humidity.
nd: No carbon dioxide release detected

Table **5-2**: The amount of ethylene released by 1 gram of seeds in 1 hour (ppm). Means ± standard error of the mean.

Species	Type	Days 0-3			Days 3-6			Days 6-9		
Setaria faberi	D	2.78	±	2.12	3.52	±	1.61	3.84	±	1.04
	M	10.92	±	0.33	6.08	±	2.45	24.58	±	8.22
Amaranthus retroflexus	D	1.25	±	1.25	0.23	±	0.23	4.17	±	2.84
	M	10.33	±	1.13	11.21	±	4.86	22.79	±	3.32
Chenopodium album	D	1.20	±	1.20	10.09	±	7.03	nd		
	M	10.54	±	1.12	8.17	±	1.09	22.83	±	4.57
Abutilon theophrasti	D	1.85	±	1.85	0.65	±	0.65	0.14	±	0.14
	M	11.50	±	0.85	7.29	±	2.33	16.42	±	7.21
Ambrosia artemisiifolia	D	2.18	±	2.18	1.85	±	1.85	2.69	±	0.89
	M	9.79	±	0.77	8.67	±	0.75	17.25	±	2.94
Setaria lutescens	D	5.51	±	1.26	1.62	±	1.36	19.12	±	13.35
	M	6.67	±	2.42	6.92	±	0.27	nd		
Echinochloa crus-galli	D	5.88	±	0.82	5.93	±	1.84	0.88	±	0.30
	M	11.33	±	1.91	5.63	±	1.39	11.17	±	5.39

D: Seeds were kept dry under ambient humidity. M: Seeds were kept moist under high humidity.
nd: No ethylene release detected

Table **5-3**: Response of adult *Harpalus pensylvanicus* beetles to volatiles released from 3 common weed seeds (dry and imbibed) and 3 chemical compounds. The p-values presented are the result of a two-sided binomial test comparing 20 beetles responses to the volatiles of interest vs. purified air.

	Imbibed		Dry	
Source of volatiles	% Response to volatiles	p-value	% Response to volatiles	p-value
Abutilon theophrasti	80	0.012	55	0.824
Ambrosia artemisiifolia	65	0.263	55	0.823
Setaria faberi	75	0.041	50	1.000
11400 ppm CO_2	15	0.003		
800 ppm CO_2	50	1.000		
33 ppb Ethylene	60	0.503		

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Chapter 6

Conclusions

The majority of weed seeds produced in agricultural fields, 68%-99%, do not grow into seedlings nor do they remain in the soil bank until the next season (Cardina et al. 1996, Gerowitt 1998). The demise of most plants is the result of being preyed upon as a seed (Nathan and Casagrandi 2004) and recent research has suggested that invertebrates are responsible for national scale regulation of weed seeds in the soil seedbank (Bohan et al. 2011). Given this important ecological and economic service in agroecosystems, it is critical to gain a better understanding of invertebrate seed predation.

Adoption of reduced tillage in agriculture has been advocated within the United States and around the globe for the many benefits associated with it (Lal 2004), including an increased number of invertebrate seed predators and the associated increases in seed predation (Baraibar et al. 2009, Brust and House 1988, Menalled et al. 2007). However, reducing tillage often means that weed control is largely accomplished through increased application of herbicides. While some researchers have developed integrated weed management strategies that control weeds and minimize herbicide application (Bates et al. 2012, Blackshaw et al. 2008, Buhler et al. 1995, Chikowo et al. 2009, Eadie et al. 1992, Hanna et al. 2000), none of these strategies have been examined in regards to their effects on weed seed predators and seed predation.

Our results suggest that a minimum tillage regime (employed by Bates et al. 2012), disturbing the upper 2-5 cm, has little negative effect on invertebrate seed predator activity-density and seed predation (Chapter 2). In my later work using the same tillage regime in a corn/soybean system with the addition of cover crops, no negatives effects on granivorous invertebrates or invertebrate seed predation were observed (Chapter 3). The integrated weed

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management system employed in this study increased the activity-density of a number of granivorous invertebrate populations when compared to no-till and organic systems and is in agreement with research suggesting that cover crops through increased cover, humidity (Clark et al. 1993, Laub and Luna 1992), and food sources increase granivorous invertebrate densities (Carmona and Landis 1999, Manley 1996, Shearin et al. 2008, Ward et al. 2011). This is in contrast to deep inversion tillage which is largely considered detrimental to agricultural invertebrates and has been shown to result in a greater than 50% decrease in granivorous invertebrates population numbers (Shearin et al. 2007).

However, seed predation rates in integrated plots did not have a corresponding increase to seed predator numbers as another study has shown (Davis and Liebman 2003). In my study, the taxa that had the strongest correlation to seed removal rates and had the largest population increase in integrated plots was Gryllidae (crickets). The omnivorous field cricket, *Gryllus pennsylvanicus* Burmeister, composed the vast majority of these captures and is known to consume common ragweed, velvetleaf, redroot pigweed, large crabgrass, and giant foxtail seeds (Brust and House 1988, Carmona et al. 1999). However, this species and *Allonemobius* sp. are also known to consume dead and alive plant matter, fruits, and both living and dead insects (Lundgren and Harwood 2012). In addition, these two taxa were shown to eat invertebrate prey when seeds were or were not present (Lundgren and Harwood 2012). Given the increase in both dead plant material and invertebrate prey in integrated plots, it seems that weed seed predation was not increased as crickets may have eaten more alternative food sources. It is reasonable to assume that omnivorous carabid species also ate more foods in addition to weed seeds. While seeming not to alter weed seed predation, increased activity-densities of omnivorous invertebrate predators may have led to increased invertebrate pest control and merits further investigation.

Although the application of a minimum tillage regime had no observed negative impacts on invertebrate seed predation rates, this tillage (i.e. vertical coulter, rotary harrow, and high

residue cultivation) buried a considerable portion ($> 42\%$) of seed surrogates below the soil surface. It is commonly believed that buried seed is not subject to predation by invertebrate granivores (Thompson 1987). However, few studies have examined weed seed predation at any soil depth. One such study by White et al. (2007) found that seed predation by *H. pensylvanicus*, one of the most abundant granivores in this study, on three types of seed was consistent at the soil surface down to the deepest depth examined, 1cm below the surface. I am aware of no studies examining invertebrate seed predation deeper than 1cm below the soil surface. More research is needed to determine what effect seed burial has on predation by granivorous invertebrates. It is possible that the min-till method applied here could lead to decreased seed predation and a higher density of seeds entering the seedbank or subsequent tillage applications may flush seeds from the soil, resulting in no additional input to the seed bank (Mulugeta and Stoltenberg 1997, Roberts and Feast 1973).

In field studies, no differences were observed in predation rates of three weed seed species, *Setaria faberi*, *Ambrosia artemisiifolia,* and *Abutilon theophrastii,* in any year, regardless of cropping system (Chapters 2,3). In contrast, laboratory studies showed small and medium-sized seeds were eaten in greater amounts than large seeds by *Harpalus pensylvanicus,* with little to no predation of large-seeded species when seeds were dry (Chapter 4). This is in agreement with a large body of literature showing seed size is a major driver of seed selection by carabids with *H. pensylvanicus* preferring medium to small-sized seeds (Honek et al. 2007, Lund and Turpin 1977, Lundgren and Rosentrater 2007, Ward 2008). However I also found that imbibition affects seed selection, and once seeds were imbibed, significantly more large seeds were eaten (Chapter 4). Given that northeastern agricultural fields receive frequent rain events, and seeds are often imbibed, it is likely that selection pressure on small and medium-sized seeds was minimized in my study, and larger seeded species were frequently eaten due to softer seed coats. In addition, it is known that carabid larvae cache weed seeds in their burrows (Hartke et

al., 1998, Kirk, 1972, Luff, 1980) and it is possible that adult beetles also exhibit this behavior. In my field studies I considered each seed removed as being predated; although, it is possible that a portion of seeds were transported to a seed cache to serve as a food source later and may never have been eaten. As such, the removal of seeds in my study may not reflect true seed predation and thus, seed selection does not occur until the time that seeds are eaten. However, this scenario seems somewhat unlikely given the results of my stable isotope study (Chapter 4). Through the marking of seeds with ^{15}N, I found that *H. pensylvanicus* beetles eat both a medium-sized seed, *S. faberi,* and a larger harder-coated seed species, *A. theophrastii,* when in their natural environment.

In addition to being seemingly easier to devour, imbibed seeds are also able to be located through olfaction. Y-tube bioassays showed that *H. pensylvanicus* adults have the ability to detect seeds when imbibed but not when dry through olfaction (Chapter 5). However, the chemical nature of the olfactory cue is unknown. It was found that the 7 weed seeds examined released both CO_2 and C_2H_4 when dry and considerably more of these chemicals when imbibed. However, neither CO_2 nor C_2H_4 elicited an attraction response from *H. pensylvanicus* adults. Nonetheless, my study suggests that the moisture level of the environment has implications for seed detection and thus the amount of weed seed entering the seed bank. In moist years, beetles may be capable of finding and eating more obscured and large seeds; while in dry years, visually obscured seeds and large seeds are less likely to be eaten and are more likely to enter the seed bank.

Since landscape position of the crop field has influence on the diversity of the carabid community (Leslie 2007), it is likely that integrated fields near a diversity of landscapes will have a larger and more diverse carabid population capable of enhanced weed control. Understanding a field's location within the landscape and the surrounding environment's role in providing a granivorous source may be critical in knowing if granivores can be an effective complementary

weed control mechanism. It would be expected that the more diverse the granivorous community around the field, the quicker granivores could respond to the increased habitat and populate the integrated fields. In this study, fields were surrounded by a diversity of fields, mowed and unmowed fence rows, and forests. As such, it would be expected that the surrounding granivorous community could quickly respond to changes in the habitat of the field and immigrate. However, integrated fields placed in a large landscape of highly tilled fields may take longer to show increases in beneficial invertebrate populations. Future research needs to better understand the role of landscape position in structuring the in-field communities of fields.

If granivores, carabids in particular, are to be an important component of an integrated weed management strategy, considerable more detail about habitat selection and food selection needs to be known. Future research further investigating the diet of beetles in their natural environment would be quite useful in understanding their role as a weed control component in integrated pest management strategies. Although the beetle species at the focus of this study are known to eat weed seeds, the proportion of seeds versus other food sources devoured is unknown and it is unknown if this varies temporally throughout the year. Larvae of beetles are rarely examined due to difficulties in capture and identification. However, it is well know that beetle larva both eat and cache seeds burrows (Hartke et al., 1998, Kirk, 1972, Luff, 1980). In order to better predict weed seed densities in integrated fields more knowledge of the above mentioned topics is critical. Growers are less apt to switch weed control strategies when much about the weed pressure their crops will face is unknown.

This study would have benefited from identification of all carabid beetle species captured in pitfall traps in field studies. While I quantified the abundant granivores, a number of less numerous granivores were not identified to species. In addition, knowing all species present would allow comparisons of beetle diversity across treatments. The field studies conducted would also have benefitted from a mark-recapture study that allowed estimates of true

invertebrate density. The use of activity-density rather than a true density estimate likely underestimated invertebrate abundance in high residue systems and overestimated it in low residue systems.

The marking of seeds with a stable isotope, ^{15}N, provided significant evidence that *H. pensylvanicus* beetles regularly ate *S. faberi* and *A. theophrastii* seeds in their natural environment. However, the study fell short of being able to determine seed predation for species in which large numbers of negative controls were not captured. Seeds were significantly enriched, greater than 10 times background rates, and yet invertebrates known to devour marked seed in the lab were only 1-3x more enriched than beetles not exposed to mark seed. As such, future studies employing a similar methodology should ensure capture of large numbers of invertebrates from the study area not exposed to mark seed (negative controls). These negative controls will allow for a conclusive determination if seed predation took place.

Diplopods are an important taxon on which to focus future seed predation investigations. Diplopods are often found to be abundant in crop fields and if granivorous may be responsible for considerable amounts of weed seed loss in agroecosystems. Using this methodology with enriched seeds would be useful in elucidating their importance and also that of carbid beetle larvae in weed seed loss. Larva are known to eat and cache weed seeds but the extent of their impact is unknown burrows (Hartke et al., 1998, Kirk, 1972, Luff, 1980).

In summary, my results indicate that the application of a minimum tillage regime (i.e. vertical coulter + rotary harrow + high residue cultivation) does not result in a decrease in overall granivorous invertebrate activity-densities and does not decrease weed species when compared to no-till. An integrated weed management system (i.e. surface tillage + cover crops) incorporating this minimum tillage regime, results in an increase in beneficial invertebrate activity-densities. The increased number of beneficial invertebrates did not result in increased weed seed predation rates but may have an effect on crop pests and merits further investigation. This min-till

approach likely buries weed seed and could potentially obscure seeds from predators but may be controlled via subsequent soil tillage operations. My study suggests that the moisture level of the environment has implications for seed detection and predation. As such, moisture level of the environment has implications for the amount of weed seed entering the seed bank. In moist years or in moist fields, beetles are capable of finding and eating more obscured seeds while in dry years and dry fields, visually obscured seeds are less likely to be found and eaten and more likely to enter the seed bank. The results presented further support a body of work showing that crickets and *H. pensylvanicus* are major seed predators. Isotopic enrichment of seeds provided a unique way to track seed predation and showed that *H. pensylvanicus* will eat large seeded weed species in an agricultural field.

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Appendix

A list of δ¹⁵N (‰) of all individual invertebrates analyzed in the stable isotope study.

Type	Taxon	Plot	$\delta^{15}N$ (‰)	Type	Taxon	Plot	$\delta^{15}N$ (‰)
- Control	*Abultion theophrastii*		8.43				
- Control	*Abultion theophrastii*		10.33	Sample	C. tricolor	F	6.62
- Control	*Abultion theophrastii*		8.46	Sample	C. tricolor	F	5.72
- Control	*Abultion theophrastii*		9.18	Sample	C. tricolor	F	7.25
- Control	*Abultion theophrastii*		9.41	Sample	C. tricolor	F	6.11
- Control	*Abultion theophrastii*		9.95	Sample	C. tricolor	V	8.18
- Control	*Abultion theophrastii*		7.74	Sample	C. tricolor	V	9.69
- Control	*Abultion theophrastii*		8.60	Sample	C. tricolor	V	8.10
- Control	*Abultion theophrastii*		7.88	Sample	C. tricolor	V	6.68
- Control	*Abultion theophrastii*		9.75	Sample	C. tricolor	V	6.22
+ Control	*Abultion theophrastii*		246.21	Sample	C. tricolor	V	8.16
+ Control	*Abultion theophrastii*		158.64	Sample	carabid larvae	F	1.81
+ Control	*Abultion theophrastii*		192.69	Sample	carabid larvae	V	3.34
+ Control	*Abultion theophrastii*		216.57	Sample	cricket	V	4.59
+ Control	*Abultion theophrastii*		127.25	Sample	cricket	V	3.83
+ Control	*Abultion theophrastii*		125.35	Sample	cricket	V	5.12
+ Control	*Abultion theophrastii*		182.15	Sample	cricket	V	6.13
+ Control	*Abultion theophrastii*		145.21	Sample	cricket	V	3.92
+ Control	*Abultion theophrastii*		111.86	Sample	Diplopoda	F	6.61
+ Control	*Abultion theophrastii*		236.11	Sample	Diplopoda	F	1.79
- Control	Setaria faberi		13.90	Sample	Diplopoda	F	3.67
- Control	Setaria faberi		12.13	Sample	Diplopoda	F	4.03
- Control	Setaria faberi		10.22	Sample	Diplopoda	F	3.67
- Control	Setaria faberi		11.69	Sample	Diplopoda	F	5.24
- Control	Setaria faberi		12.17	Sample	Diplopoda	F	1.87
- Control	Setaria faberi		11.81	Sample	Diplopoda	V	2.32
- Control	Setaria faberi		10.27	Sample	Diplopoda	V	0.92
- Control	Setaria faberi		10.50	Sample	Diplopoda	V	2.67
- Control	Setaria faberi		11.56	Sample	Diplopoda	V	3.37

Appendix

- Control	Setaria faberi	10.27	Sample	Diplopoda	V	2.76
+ Control	*Setaria faberi*	206.95	Sample	European earwig	V	2.62
+ Control	*Setaria faberi*	146.43	Sample	F. auricularia	V	5.13
+ Control	*Setaria faberi*	314.00	Sample	H. affinis	V	5.45
+ Control	*Setaria faberi*	183.96	Sample	H. affinis	V	5.95
+ Control	*Setaria faberi*	102.26	Sample	H. herbivagus	V	3.67
+ Control	*Setaria faberi*	240.47	Sample	H. herbivagus	V	4.02
+ Control	*Setaria faberi*	243.59	Sample	H. pensylvanicus	F	5.78
+ Control	*Setaria faberi*	229.96	Sample	H. pensylvanicus	F	2.04
+ Control	*Setaria faberi*	177.59	Sample	H. pensylvanicus	F	2.38
+ Control	*Setaria faberi*	149.79	Sample	H. pensylvanicus	F	2.66
- Control	Araneae	7.89	Sample	H. pensylvanicus	F	3.12
- Control	Araneae	8.48	Sample	H. pensylvanicus	F	3.28
- Control	Araneae	8.01	Sample	H. pensylvanicus	F	3.44
- Control	Araneae	8.27	Sample	H. pensylvanicus	F	3.60
- Control	Araneae	9.54	Sample	H. pensylvanicus	F	3.69
- Control	Araneae	10.74	Sample	H. pensylvanicus	F	3.81
- Control	Araneae	8.06	Sample	H. pensylvanicus	F	3.85
- Control	Araneae	8.76	Sample	H. pensylvanicus	F	3.86
- Control	Araneae	8.49	Sample	H. pensylvanicus	F	3.95
- Control	Araneae	8.00	Sample	H. pensylvanicus	F	3.99
- Control	Araneae	8.36	Sample	H. pensylvanicus	F	4.00
- Control	Araneae	7.37	Sample	H. pensylvanicus	F	4.26
- Control	Araneae	7.50	Sample	H. pensylvanicus	F	4.41
- Control	Araneae	7.56	Sample	H. pensylvanicus	F	4.45
- Control	Araneae	7.46	Sample	H. pensylvanicus	F	4.54
- Control	Diplopoda	3.52	Sample	H. pensylvanicus	F	4.71
- Control	Diplopoda	2.30	Sample	H. pensylvanicus	F	4.81
- Control	Diplopoda	3.48	Sample	H. pensylvanicus	F	4.95
- Control	Diplopoda	2.79	Sample	H. pensylvanicus	F	5.37
- Control	Diplopoda	2.43	Sample	H. pensylvanicus	F	5.81
- Control	Diplopoda	3.75	Sample	H. pensylvanicus	F	6.31
- Control	Diplopoda	3.90	Sample	H. pensylvanicus	F	6.54
- Control	Diplopoda	3.75	Sample	H. pensylvanicus	F	9.06
- Control	Diplopoda	3.77	Sample	H. pensylvanicus	F	9.47
- Control	Diplopoda	4.16	Sample	H. pensylvanicus	F	9.79
- Control	Diplopoda	5.06	Sample	H. pensylvanicus	F	10.18
- Control	Diplopoda	3.68	Sample	H. pensylvanicus	F	12.55
- Control	Diplopoda	5.06	Sample	H. pensylvanicus	V	2.15
- Control	Diplopoda	5.47	Sample	H. pensylvanicus	V	2.28

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- Control	Diplopoda		4.06	Sample	H. pensylvanicus	V	2.56
- Control	*H. pensylvanicus*		5.56	Sample	H. pensylvanicus	V	3.27
- Control	*H. pensylvanicus*		3.33	Sample	H. pensylvanicus	V	3.34
- Control	*H. pensylvanicus*		4.50	Sample	H. pensylvanicus	V	3.45
- Control	*H. pensylvanicus*		4.61	Sample	H. pensylvanicus	V	3.52
- Control	*H. pensylvanicus*		1.70	Sample	H. pensylvanicus	V	3.54
- Control	*H. pensylvanicus*		6.83	Sample	H. pensylvanicus	V	3.63
- Control	*H. pensylvanicus*		5.77	Sample	H. pensylvanicus	V	3.64
- Control	*H. pensylvanicus*		5.58	Sample	H. pensylvanicus	V	3.74
- Control	*H. pensylvanicus*		4.66	Sample	H. pensylvanicus	V	3.74
- Control	*H. pensylvanicus*		4.22	Sample	H. pensylvanicus	V	3.96
- Control	*H. pensylvanicus*		4.60	Sample	H. pensylvanicus	V	4.05
- Control	*H. pensylvanicus*		3.40	Sample	H. pensylvanicus	V	4.10
- Control	*H. pensylvanicus*		4.90	Sample	H. pensylvanicus	V	4.21
- Control	*H. pensylvanicus*		4.25	Sample	H. pensylvanicus	V	4.45
- Control	*H. pensylvanicus*		4.11	Sample	H. pensylvanicus	V	4.73
- Control	*H. pensylvanicus*		3.86	Sample	H. pensylvanicus	V	4.81
- Control	*H. pensylvanicus*		4.10	Sample	H. pensylvanicus	V	4.83
- Control	*H. pensylvanicus*		3.15	Sample	H. pensylvanicus	V	4.83
- Control	*H. pensylvanicus*		3.91	Sample	H. pensylvanicus	V	4.95
- Control	*H. pensylvanicus*		4.85	Sample	H. pensylvanicus	V	5.23
+ Control	*H. pensylvanicus*	F	14.02	Sample	H. pensylvanicus	V	5.34
+ Control	*H. pensylvanicus*	F	6.27	Sample	H. pensylvanicus	V	5.34
+ Control	*H. pensylvanicus*	F	24.11	Sample	H. pensylvanicus	V	6.23
+ Control	*H. pensylvanicus*	F	7.09	Sample	H. pensylvanicus	V	6.32
+ Control	*H. pensylvanicus*	F	15.93	Sample	H. pensylvanicus	V	6.52
+ Control	*H. pensylvanicus*	F	20.25	Sample	H. pensylvanicus	V	6.66
+ Control	*H. pensylvanicus*	F	19.75	Sample	H. pensylvanicus	V	7.27
+ Control	*H. pensylvanicus*	V	6.31	Sample	H. pensylvanicus	V	9.37
+ Control	*H. pensylvanicus*	V	7.43	Sample	P. lucublandus	V	6.86
+ Control	*H. pensylvanicus*	V	6.06	Sample	P. melanarius	F	7.23
+ Control	*H. pensylvanicus*	V	6.31	Sample	P. melanarius	F	7.06
+ Control	*H. pensylvanicus*	V	6.38	Sample	P. melanarius	F	6.89
+ Control	*H. pensylvanicus*	V	5.73	Sample	P. melanarius	F	7.60
+ Control	*H. pensylvanicus*	V	6.27	Sample	P. melanarius	F	6.34
+ Control	*H. pensylvanicus*	V	5.76	Sample	P. melanarius	F	6.67
Sample	A. nasatum	V	7.56	Sample	P. melanarius	F	7.97
Sample	Amara sp.	V	4.11	Sample	P. melanarius	V	7.52
Sample	Amara sp.	V	3.24	Sample	P. melanarius	V	7.28
Sample	Araneae	F	8.11	Sample	P. melanarius	V	7.72

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Sample	Araneae	F	7.00	Sample	P. melanarius	V	6.87
Sample	Araneae	F	7.23	Sample	P. melanarius	V	5.51
Sample	Araneae	F	7.98	Sample	P. melanarius	V	8.47
Sample	Araneae	F	9.23	Sample	P. melanarius	V	9.25
Sample	Araneae	V	9.16	Sample	P. melanarius	V	8.02
Sample	Araneae	V	7.94	Sample	P. mutus	F	7.02
Sample	Araneae	V	8.36	Sample	P. mutus	V	6.93
Sample	Araneae	V	7.94	Sample	P. opilio	V	8.11
Sample	B. quad Opp	V	8.23	Sample	P. stygicus	F	9.46
Sample	C. tricolor	F	5.96	Sample	P. stygicus	F	8.02
Sample	C. tricolor	F	6.62	Sample	P. stygicus	F	7.94
Sample	C. tricolor	F	7.19	Sample	weevil	V	4.36

F = Captured in plots with enriched giant foxtail applied to the soil surface, V = Captured in plots with enriched velvetleaf applied to the soil surface. Sample refers to a field collected specimen that was captured from a plot with the addition of isotopically enriched seeds.

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