

# Self-regulating weed control through the enhancement of beneficial ground dwelling invertebrates

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## Project Information

### Summary:

The objective of this research project was 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 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 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,  $^{15}\text{N}$ , it was shown that *Harpalus 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. This research shows that integration of cover crops into a reduced 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.

## Introduction:

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  $\text{m}^{-2} \text{day}^{-1}$  (Honek et al. 2003) and crickets (Gryllidae) often the most abundant seed predators (O'Rourke et al. 2006).

### 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.

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.

### 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).

### 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. 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. Residues on the soil surface can be beneficial to carabid beetles. 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.

### Project Objectives:

#### Objective 1

Determine if the integration of legume green manure crops into reduced tillage cropping systems will enhance the diversity, abundance, and activity of the beneficial ground dwelling invertebrate community, and thereby the pest management services with respect to weed seed predation.

This objective was completed and it was shown that 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. The reduced-till organic plots were not viable and data was not collected from them as proposed. The weed density was significantly higher than in other treatments and in 2010 it was decided to cease data collection in these plots.

#### Objective 2

Determine if weed seed 'debts' from the soil seed bank by invertebrate seed predators are weed species specific, thereby facilitating a shift in the weed community composition.

All proposed weed seed preference studies with *Harpalus pensylvanicus* were complete. However, *Harpalus pensylvanicus* was the only species abundant in the fields and therefore no other beetles were caught in large enough numbers to perform preference studies. In addition to the work proposed, a study using a y-tube olfactometer was conducted to determine if beetles could sense visually obscured imbibed seed and/or visually obscured dry seeds. In addition, cafeteria studies were conducted with imbibed seed in addition to the dry seed proposed.

Another study was completed to elucidate the granivorous role of invertebrates in agroecosystems that was not proposed. Velvetleaf (*Abutilon theophrastii*) seeds were grown in the laboratory and marked with the stable isotope,  $^{15}\text{N}$ . These seeds were then distributed in a field. Invertebrates were captured in the field in the subsequent week and analyzed to determine the amount of  $^{15}\text{N}$  in their bodies and if they had eaten marked weed seeds. The study was also repeated with giant foxtail (*Setaria faberi*) seeds.

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## Research

### Materials and methods:

#### Field Study

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.

A hairy vetch (*Vicia villosa*) cover crop was planted in integrated and organic plots in the fall of 2009. The vetch was chemically terminated before planting in May 2010 with a burndown herbicide program comprised of 0.84 kg ae ha<sup>-1</sup> plus 0.28 kg ae ha<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> s-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl]acetamide], 0.19 kg ai ha<sup>-1</sup> mesotrione [2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione], and 1.54 kg ai ha<sup>-1</sup> 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<sup>-1</sup> nicosulfuron [2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide], 0.0123 kg ai ha<sup>-1</sup> rimsulfuron [N-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide], 0.84 kg ha<sup>-1</sup>

atrazine, and 0.28 kg ha<sup>-1</sup> mesotrione. Herbicides were applied in water at 187 L ha<sup>-1</sup> at 207 kPa.

The integrated treatment received the application of a vertical coultter + double pull rotary harrow in place of a pre-emergent herbicide program prior to planting in May 2010. The vertical coultter has a series of fluted coultters that enter the first few centimeters of soil to provide increased air exchange and increase soil temperatures. A vertical coultter is commercially available from Great Plains Mfg Inc. and is known as the Turbo-Till®. The vertical coultter 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 cultmulcher. Post-emergent weeds were controlled with two applications of a rotary hoe in early-mid June and subsequently, two applications of a cultivator.

Following corn harvest in early November 2010, winter rye was planted as a cover crop in organic and integrated plots. Invertebrates activity-density was assessed via pitfall trapping from July thru September in 2010 and from July to October in 2011. In addition, 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 enclosure. *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<sup>2</sup>. Seed predation was quantified by counting remaining seeds after 168 hours in the field.

### Seed Selection

Thirty adult *H. pensylvanicus* beetles were individually placed in containers with 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. 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.

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, <sup>15</sup>N-Ammonium Nitrate, as outlined by Carlo et al. (2009).

In September 2011, 73g of collected *A. theophrasti* seeds were spread in 3 plots, 3m<sup>2</sup>, each separated by 20m in a no-till field. Likewise, 55g of *S. faberi* seeds were

placed in 3, 3m<sup>2</sup> 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 to capture invertebrates 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  $\delta^{15}\text{N}$  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 analyzed for isotopic content to determine if marked seed had been eaten.

#### Beetle Olfaction

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 analyzed for volatile organic compound release at two seed hydration states: 1) imbibed at 100% RH and 2) ambient dry. Bioassays were conducted with adult *H. pensylvanicus* beetles (Coleoptera: Carabidae) to determine if they show behavioral responses to dry or imbibed seeds.

### Research results and discussion:

#### Field Study

Crop yield was not significantly different between the three plot types (Table 1). In both 2010 and 2011, the most abundant granivores were *H. pensylvanicus* and members of 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 integrated crop management system (i.e. surface tillage + cover crops) resulted in an increase in beneficial invertebrate activity-densities (Figure 1, Figure 2). The increased number of beneficial invertebrates did not result in increased weed seed predation rates (Figure 3).

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, unpublished data) 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).

#### Seed Selection

*H. pensylvanicus* ate significantly more imbibed than dry seed of all seed species (One-way ANOVA,  $p < 0.001$ ) (Figure 4). 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).

Adult *H. pensylvanicus* beetles and diplopods ate enriched weed seeds. 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 (14.3%), with no obviously enriched individuals out of the 5 individuals from enriched velvetleaf plots.

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.

#### Beetle Olfaction

Two volatile organic compounds were found to be released by seeds before germination, carbon dioxide and ethylene. 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,  $p > 0.05$ ). Beetles did not show a significant response to any of the 3 seed species when dry (binomial,  $p > 0.05$ ). 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. This suggests that 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.

- [Figure 1](#)
- [Table 1](#)
- [Figure 4](#)
- [Figure 2](#)
- [Figure 3](#)

#### Research conclusions:

There has been a strong emphasis nationwide to move away from conventional tillage systems due to the negative impact tillage can have on soil quality and erosion potential (reviewed by Blevins et al. 1998). Less tillage often requires greater reliance on herbicides, which can have a negative impact on the quality of surface and ground waters (Gillion and Hamilton 2006), and can lead to the development of herbicide resistant weed communities (Heap 2009). In contrast to these negative environmental consequences of reduced tillage, there are aspects of reduced tillage systems that are compatible with sustainable pest management.

This project illustrates that a reduced tillage system can enhance the granivorous invertebrate community while effectively controlling weeds with a focus on soil and water conservation. Such integrated crop management systems will benefit farmers in the Northeast region by providing robust and cost-effective pest, crop and soil management, and benefit the non-agriculture stakeholders of the region with improved environmental quality.

## **Participation Summary**

# Education & Outreach Activities and Participation Summary

## **PARTICIPATION SUMMARY:**

Education/outreach description:

Two publications presenting this research are being prepared for submission to nationally recognized journals. Three poster presentations have been presented concerning this research. One presentation was at the Agricultural Sciences Research Expo, University Park, PA, and the other two at the Penn State Graduate Research Exhibitions in 2010 and 2011. In 2010, I was awarded a first place prize at the exhibition for the work and its presentation. Since 2010, three oral presentations have been presented on this research at Pennsylvania State University.

Law, J.J. 2012. Granivorous Invertebrates and Weed Seed Predation: An Ecological Approach to Weed Management. Ph.D. Dissertation, Pennsylvania State University, State College, PA.

## Project Outcomes

Project outcomes:

### Farmer Adoption

The research completed shows considerable promise for future application. The integrated reduced tillage system utilized had equal crop yields with fewer inputs than other cropping systems. In addition, field and laboratory work examining granivorous beetles showed them to have the potential to effectively find and predate weed seeds. However, no specific recommendations can be made to growers without further research. Future research is needed to determine the effectiveness of these granivores over the long-term and if they remain effective in a diversity of areas and environmental conditions.

Assessment of Project Approach and Areas of Further Study:

### Areas needing additional study

1. It is important to understand why additional seed predators did not result in additional seed predation. Given the increase in both dead plant material and invertebrate prey in integrated plots, it seems possible that weed seed

predation was not increased as invertebrates may have eaten more alternative food sources.

Likewise, 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.

More research is needed to determine what effect seed burial has on predation by granivorous invertebrates. 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. If granivores can locate buried seed then they are likely capable of being more effective components of a weed control strategy.

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.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture or SARE.



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