

**SEED PREDATION BY INVERTEBRATES: METHODOLOGY
AND ASSESSMENT OF REMOVAL RATES IN THE
TRANSITION TO AN ORGANIC CASH GRAINS SYSTEM**

A Thesis

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ABSTRACT

In the development of sustainable agricultural systems, it is increasingly clear that chemical control of weeds must be replaced with effective ecologically-based weed control measures. As part of this effort, seed predators and the associated ecosystem service of weed seed predation were assessed in the transition to an organic cash grains system. Invertebrate activity density was higher in the spelt/clover than in the corn plots, where ‘moderate’ predation of seeds from all three weed species was highest. By contrast to giant foxtail and common lambsquarters, large-seeded velvetleaf had more instances of ‘moderate’ removal with vertebrate access ($P < 0.01$). Invertebrates, however, were primarily responsible for the 20% of samples in 2006 showing ‘extensive’ (31-100%) velvetleaf removal ($P < 0.001$).

A standardized methodology for evaluating seed predation by invertebrates is needed to increase our capacity to generalize across studies. This work may facilitate transitioning beyond simple ecosystem services assessments into providing a practical set of recommendations for agricultural managers that enhance invertebrate weed seed predation. In a second study, we compared the effect on seed predation of two common methods of offering weed seeds to potential predators: the sandpaper and soil substrate methods. Results suggest caution in using synthetic substrates, such as sandpaper, when assessing predation of small-seeded species ($>1 \text{ mg seed}^{-1}$), or when seed predators are predominantly invertebrates. By contrast, predation of the larger-seeded velvetleaf and giant foxtail were less affected by sampling method, perhaps due to greater removal by vertebrates.

As a corollary to both studies, we investigated the use of geospatial analysis to estimate spatial autocorrelation of invertebrate populations and seed removal rates. Geospatial analysis revealed significant spatial autocorrelation of insect activity density with the location of clustering varying by insect species and sampling month,

with seed removal rates sometimes similarly autocorrelated. Without investigating this spatial component, several instances of strong positive correlation between a spatially autocorrelated invertebrate species and removal of a species of weed would have been unobserved. Failure to consider the clustering in insect populations and associated seed removal rates can cause important effects and/or trends to be masked when averaged across an entire system.

BIOGRAPHICAL SKETCH

Rachel E. Shuler received her B.A. from Oberlin College with a major in English literature and a minor in Spanish. She decided to pursue a PhD in the sciences rather late in her undergraduate career after spending her junior and senior years working with a professor of Botany, Dr. Marta Laskowski, on the influence of auxin on lateral root formation in *Arabidopsis thaliana*. They grew mutants of those genes whose cognate mRNAs were more abundant after auxin treatment in order to understand their role in the complicated physiological responses attributed to this hormone.

After graduating, she received an NSF REU grant to work with an entomologist at the University of Virginia, Dr. T'ai Roulston, designing and implementing a project to investigate the relationship between farming practices and the diversity and abundance of native pollinators. She focused in particular on the activity density of the squash bee *Peponapis pruinosa*, the specialist pollinator of squash, pumpkins, zucchini, and other members of the genus *Cucurbita*. Before coming to Cornell, she also lived for several months in the Sarapiquí region of Costa Rica and worked with a MS student on a survey of herpetofauna on farm woodlots and on the climax forest of the La Selva Biological Preserve and Research Station.

The invertebrate ecosystem assessment work first experienced in the squash bee research has continued at Cornell University in two distinct multidisciplinary research projects. In both she partnered with researchers to assess invertebrate populations, especially carabid beetles, and associated post-dispersal weed seed predation.

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**1. POST-DISPERSAL WEED SEED REMOVAL INCREASES IN THE
TRANSITION FROM CONVENTIONALLY MANAGED CORN TO AN
ORGANIC CASH GRAIN SYSTEM**

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Abstract

In the development of sustainable agricultural systems, it is increasingly clear that chemical control of weeds must be replaced with effective ecologically-based weed control measures. As part of this effort, seed predators and the associated ecosystem service of weed seed predation were assessed in the transition to an organic cash grains system. Two different entry points, and four distinct weed and nutrient treatments in the three-year rotation of corn/soybean/winter spelt (overseeded with clover) were sampled over the first two years of the transition (2005, 2006). Seed predation was assessed for velvetleaf, giant foxtail, and common lambsquarters using feeding trials of 40 seeds each offered during a 48-hour period. Year was the largest source of variation, with an average of 1.6 times greater instances of seed predation occurring in all months of 2006 relative to 2005 ($P < 0.001$). Geospatial analysis revealed significant spatial autocorrelation of insect activity density with the location of clustering varying by insect species and sampling month. Seed removal rates were not similarly autocorrelated. No differences in invertebrate activity or seed predation were observed between organic weed and nutrient treatments. Invertebrates were primarily responsible for instances of 'moderate' seed predation of giant foxtail and common lambsquarters (1-28% removal) but vertebrate access doubled instances of 'extensive' predation (31-100% removal) ($P < 0.001$). Invertebrate activity density was highest in the spelt/clover plots, where 'moderate' predation of seeds from all three weed species was the highest. By contrast to giant foxtail and common lambsquarters, large-seeded velvetleaf had more instances of 'moderate' removal with vertebrate access ($P < 0.01$). Invertebrates, however, were primarily responsible for the 20% of samples in 2006 showing 'extensive' (31-100%) velvetleaf removal ($P = 0.54$). This finding was counterintuitive given that 78% of 'extensive' velvetleaf

removal measured using identical methods in the adjacent conventional corn study occurred with vertebrate access. ‘Extensive’ invertebrate velvetleaf removal also occurred equally in the soybean plots and spelt/clover mixture plots, perhaps indicating that the invertebrates responsible for high removal rates – likely the seed specialists *Harpalus caliginosus* or *H. rufipes* captured at high rates in this system - may have greater daily dispersal distances than those invertebrate predators responsible for only ‘moderate’ seed removal.

Nomenclature: Common lambsquarters, *Chenopodium album* L. # CHEAL; Giant foxtail, *Setaria faberi* Herrm. SETFA; Velvetleaf, *Abutilon theophrasti* Medicus # ABUTH; clover *Trifolium pratense* L.; corn, *Zea mays* L.; spelt *Triticum spelta* L.; soybean, *Glycine max.* (L.) Merr; *Harpalus caliginosus* Fab.; *Harpalus rufipes* Degeer.

Key words: Biological control, Carabidae, cash grains, ground beetle, organic systems, seed bank dynamics, seed predators, seed predation, transition to organic.

Introduction

Development and implementation of ecologically based preventative weed management techniques are essential to reducing our reliance on therapeutic weed control measures, particularly herbicide application (Jordan 1996, Liebman et al. 2001). In agriculture, costs associated with weeds are greater than the combined impact of both diseases and insect pests (Bridges 1992). Weed control and other damages, including lowered crop yields, are conservatively estimated at between \$15 and \$26 billion annually in the U.S. (Bridges 1992; Pimentel et al. 2000, respectively). One emerging area of ecologically-based weed management focuses on how farm management practices affect the diversity and abundances of seed predator assemblages (Gallandt et al. 2005; Menalled et al. 1999; O'Rourke et al. 2005; Westerman et al. 2003). A more in depth understanding of invertebrate population dynamics may not only lead to more effective weed suppression (Briese 2000; Janzen 1969; Julien and Griffiths 1998; Zhang et al. 1997), but can be useful for assessing habitat quality (Blake et al. 2003).

The most obvious context for ecological weed management is within organic cropping systems. A major focus of these systems is to optimize ecosystem stability, resilience, and to enhance species diversity and richness as a way of promoting ecosystem services. However, weeds are considered the foremost pest problem in organic/lower input row cropping systems (Bond and Grundy 2001; OFRF 1999). Even organic farms that are considered bio-physically mature and highly efficient

organic operations appear to lose some yield to weed competition in 25% of their crops (Mohler, unpublished data).

Weed problems are often significant in the transition from conventional to organic agriculture, because weed populations can no longer be suppressed using herbicides and the grower has not yet become skilled at implementing alternative strategies (Dabbert and Madden 1986; Delate and Cambardella 1994). The difficulty of suppressing weeds in lower input systems, particularly during transition periods, may be a key factor limiting the adoption of organic practices (Bond and Grundy 1998). Therefore, any strategy or factor that reduces weed pressure in the transition to organic systems may be especially valuable. Enhancing the post-dispersal predation of weed seeds by vertebrates and invertebrates may be one strategy to achieve this goal.

Levels of post-dispersal seed predation are difficult to quantify and challenging to correlate precisely with vertebrates or invertebrates within a system. In general, vertebrates tend to be more consistent predators during the growing season, while invertebrates have a more variable seasonal phenology (Marino et al. 1997; Westerman et al. 2003). The relative importance of invertebrates and vertebrates as weed seed predators varies greatly by study system, but invertebrates can be the dominant source of weed seed removal (Brust and House 1998; Cromar et al. 1999; Gallandt et al. 2005; Honek et al. 2003). This potential for extensive weed seed removal makes it especially critical to determine which agricultural systems and management practices are most hospitable to beneficial invertebrate seed predators.

Carabid beetles (Coleoptera: Carabidae) are some of the most abundant generalist invertebrate seed predators (Allen 1979; Aviron et al. 2005; Cole et al. 2005; Ellsburly

et al. 2005; French 2004; Luff 1987; Thiele 1977). These are highly predatory beetles that have the potential to maintain a variety of insect pests below outbreak levels in annual crops (Menalled et al. 1999). In addition to consuming insect pests, carabids also readily consume seeds and seedlings and have been cited as major weed seed predators in many agricultural systems (Andersson 1998; Brandmayr and Brandmayr 1977; Cardina et al. 1996; Singer et al. 2000).

Carabids may be representative of a large class of invertebrate seed predators since their life history suggests that they are particularly responsive to agricultural management practices. In particular, soil type, drainage class, litter depth, soil moisture content, organic content, and pH have been shown to affect the distribution and abundance of these beetles (Eyre 1994; Petit and Usher 1998). Carabid beetle activity density is often many times greater in no-till or conservation tillage systems relative to conventional tillage systems (House and All 1981; House and Parmalee 1985). This trend may apply to a broad spectrum of economically important invertebrates. For instance, ground-nesting bees essential for crop pollination are also more abundant under conservation tillage systems compared with conventional systems (Shuler et al. 2005).

Management practices can also alter the potential for predation by changing seed and other resource availabilities within a system. Seed burial is a major determinant of predation rates with increased seed survival often being associated with burial (Crawley and Long 1995). Burial is a particularly important factor affecting seed predation levels in cropping systems because different tillage and cultivation methods typically result in burial of seeds at varying depths (Mohler et al. 2006). Given the

relatively high longevity of many weed seeds, fluctuating levels of predation may have little effect on aboveground plant abundance if sufficient recruitment from the seedbank occurs (Crawley 1990).

Organic and lower-input production systems are usually able to sustain a greater abundance of invertebrates than conventional systems. Pfiffner and Niggli (1996) reported carabid densities in organic and biodynamic systems that were double those found in conventional systems. These differences were attributed to the effects of reduced pesticide use, greater ground cover, and increased use of compost and organic soil amendments in the organic and biodynamic systems. Pesticide application, especially broad-spectrum pesticides, and conventional tillage practices, often reduce the abundance of carabid beetles (Brust 1994; House and Parmelee 1985; Stinner and House 1990).

Our two-year study focused on the biologically and economically difficult transition period from conventional to organic crop management. We assessed invertebrate populations and associated weed seed removal rates in a transitional organic three-year rotation of corn (*Zea mays* L.) soybean (*Glycine max.* (L.) Merr. and spelt (*Triticum spelta* L.) overseeded with red clover (*Trifolium pratense* L.).

To quantify the contribution of seed predation to weed seed dynamics in this transition, we established five hypotheses: (1) estimates of seed predation provided by choice feeding assays are positively correlated with measured activity density of invertebrate seed predators; (2) seed predation rates are highest in the transitional organic system because of greater ground cover and landscape complexity by contrast to conventionally managed plots (3) invertebrate activity-density is greatest in those

crops of the rotation with the most ground cover (notably the spelt-clover mixture) and in those treatments with the least soil disturbance (the conventionally managed controls); (4) invertebrate activity-density is greatest later in the growing season and increases in the second year of the transition to organic management

Materials and Methods

Field Site and Experimental Design

This study was carried out during the 2005 and 2006 growing seasons at the Robert Musgrave Research Farm of Cornell University near Aurora, NY (42°44' N, 76°39' W). The soil at the field site was a Lima silt loam (fine-loamy, mixed, mesic Glossoboric Hapludalf). This study was part of an ongoing larger project investigating the possible benefits of various pest and nutrient management techniques in the transition from a conventional to an organic cash grain system.

We used a modified randomized block design with five cropping system treatments (4 transitional organic + 1 conventional control treatment), two entry points in the three-year rotation, and 4 replications (total of 40 plots). Treatment plots were 12.1 by 36.4 m. The three-year rotation consisted of soybean followed by winter (year 1) spelt over-seeded with red clover (year 2) and corn (year 3). Entry point A began with soybean in 2005 and entry point B began with corn in 2005. The field site was conventionally managed for corn in 2004 prior to the start of the study.

Each entry point received four distinct treatments designed from best practices of exemplary organic growers. These treatments can be summarized as: (1) high nutrient input to overcome potential N, P and K limitation in spelt and corn. Cultivation involved tine weeding (2 X) followed by the row crop cultivator (2 times); (2) reduced

input to maximize profit; (3) intensive weed management; (4) ridge tillage, with ridges built initially in row crops by cultivation. This treatment reduced tillage intensity and allowed for controlled wheel traffic to maintain soil quality; and (5) conventionally managed control, using Cornell recommended practices (Cornell Cooperative Extension, 2005). This treatment was physically isolated 50 ft (15.2 m) from the other four treatment plot areas in order to preserve the transitional organic status of the field site (Figure 1.1).

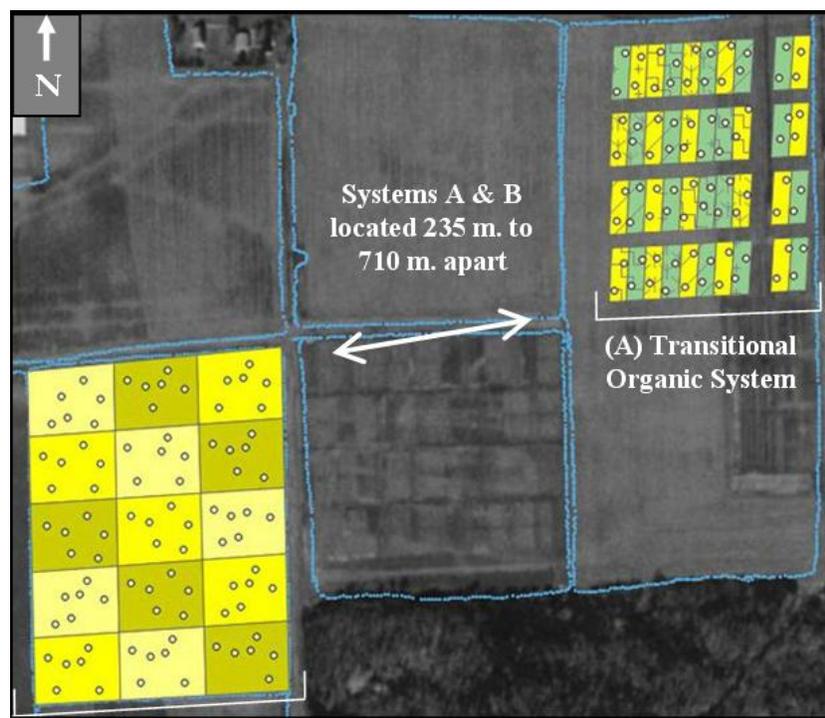


Figure 1.1. Aerial view of organic transitional field site (A) and adjacent conventionally managed corn field site (B) at Cornell University's Robert Musgrave Research Farm in Aurora NY

Assessing Seed Predation

Seed predation rates were determined for three annual weeds, common lambsquarters, giant foxtail, and velvetleaf. These are three of the most common and troublesome agricultural weeds in New York (Bridges 1992; Hartzler et al. 1993;

Singer et al. 2000). Seeds were collected on site at the Robert Musgrave Research Farm in 2003 and 2004 and stored in paper bags at 3.5 C to inhibit germination and maintain long-term viability. The wide range in mean seed size of these three species (7.3 mg for velvetleaf, 1.6 mg for giant foxtail, and 0.70 mg for common lambsquarters) may have been helpful in attracting different feeding clades of weed seed predators.

The seed predation protocol used in this study was a modified version of the procedure used by Westerman et al. (2003) and O'Rourke et al. (2006). Sandpaper with maroon/brown backing¹ was cut in half to 23 by 14 cm rectangles. Adhesive² was sprayed lightly and evenly over the surface of the sandpaper card for 2 s at a distance of approximately 30 cm. Forty seeds each of the three target weed species were placed evenly over the sandpaper surface, but avoiding 1 cm closest to the card edges. A small glass jar was gently rolled over the card to increase surface area contact of the larger velvetleaf seeds with the adhesive. A fine layer of seed-free soil was then dusted over the entire card surface using a large chef's canister with a perforated lid designed for dusting spices. After 30 min., the adhesive was dry and cards were carefully placed in plastic bags for transport to the field. Cards were positioned flush with the soil surface with corners weighted by small fieldstones. Any seeds loosened during transport were caught in the ziplock bag and were dusted over the surface of the installed card. Cards did not curl or bend and were never exposed to rainfall. Seeds did not germinate during the 48 hour window in the field. After sampling, the cards were placed in bags for transport. In the lab, seeds were brushed off of the sandpaper into a 3mm sieve to separate larger field debris, separated from fine soil by agitation in a 0.5 mm sieve, and finally counted by hand.

The seed density of 40 seeds of each weed species on the sandpaper card was 1000 seeds m⁻², with a combined seed density of 3000 seeds m⁻², levels typical of temperate

cropping systems with moderate weed seed production. The suitability of the seed density used was confirmed by on site sampling of the surface-available seedbank (data not shown). This total seed density was comparable to or less than that used in similar experiments (Brust and House 1988; Cardina et al. 1996; Cromar et al. 1999), and far lower than the combined density of 25,200 seeds m⁻² used by Gallandt (2005). Since seed predation may be density dependent, using a seed density typical of our system may reduce density-dependent inflation of predation rates (Cardina et al. 1996; Cromar et al. 1999).

Sampling was conducted once monthly from June-to-September during a 48-h window beginning between 9 and 11 A.M. Eastern Daylight Time (EDT). Each of the 40 plots had a seed predation card sited randomly in the northern and southern half of the plot adjacent to an insect pitfall for a total of 80 cards each sampling period. To avoid seed loss during rainfall events, sampling was carried out when less than a 20% probability of rain was forecast. Samples were discarded if any precipitation occurred during the 48-h window. These precipitation restrictions resulted in a total of four and two samplings conducted during the 2005 and 2006 seasons, respectively.

To quantify the portion of target seeds removed that could be attributed to vertebrates rather than ground-dwelling arthropods, vertebrate exclosures were fitted on half the seed predation assays established during each sampling period. The vertebrate exclosures consisted of a 30 by 30 by 10 cm cap constructed from 0.5-cm wire mesh allowing the passage of beetles and other invertebrates but not small mammals. In preliminary work, no differences in foraging behavior were observed for invertebrates and vertebrates in the presence of these caged exclosures (data not shown).

To calculate experimental error associated with this sampling method, 1.5- by 1.5-mm window screen was affixed over the vertebrate exclosure to deny all predators

entry. At each sampling period, total exclosures were placed over ten sandpaper predation substrate setups and processed to determine the percentage seed loss associated with sampling error rather than predation.

Invertebrate Pitfalls

Within the 40 plots, one pitfall was randomly placed in the northern and southern half of each plot. Pitfall traps consisted of a plastic cup (9 cm upper diameter by 12 cm tall) buried with the upper rim flush with ground level, and a smaller inner cup (7.5 by 4.5 cm) serving as a lining for easy specimen removal. Once monthly, and depending on weather conditions, pitfalls were uncovered, inner cups filled with ethylene glycol were inserted and open for a 48-h period. After this period, arthropods within the pitfalls were washed in water to remove the ethylene glycol, identified, and transferred to vials filled with 70% ETOH. Invertebrate activity density was assessed using simple linear contrasts on $\log(Y+1)$ transformed data.

Conventionally Managed Corn Study

We conducted research in both the organic transitional rotation study and conventionally managed corn study simultaneously in 2005 and 2006 using identical seed predation and invertebrate pitfall and processing methodologies. These two field trials were located 235- to-710 m apart at their nearest and farthest points, respectively (Figure 1.1). Comparison of seed predation levels or invertebrate activity densities between the two studies are only useful for determining broad generalizations about seasonal effects on these parameters because they were not planned as part of either experimental design.

Statistical Analysis

Seed predation rates were coded as dummy variables for PLUM ordinal regression with a negative log-log or probit link function (determined by dependant variable distribution) using SPSS for Windows³. The CROSSTABS procedure in SPSS was used to perform contingency table analysis on ordinal seed removal rates and vertebrate exclosure, year, month of sampling, replicate, entry point in the three-year rotation, the four weed/nutrient management treatments, and spatial location of plot in the field. Statistical significance was accepted at the 0.01 alpha level or less using the Bonferroni correction to reduce the possibility of type I error from multiple comparisons.

Ordinal categories for seed removal of each weed species were coded as: 0 = no predation, 1 = moderate seed predation (1-10 seeds removed or 1-28% removal, excluding seeds lost to experimental error), 2 = extensive seed predation (10+ seeds removed or 31-100% removal). These categories were determined after assessing experimental error associated with each method, as indicated by the control treatment that denied access to each seed predation setup to all predators. Seed loss due to processing and other sources of experimental error varied by method and by seed size of weed species. The range of experimental error across seed types included an error of +/- 1 for large-seeded velvetleaf to an error of +/- 3 seeds associated with the small-seeded common lambsquarters.

A final dependant variable was constructed by adding the ordinal variables from all three weed species into one composite value. This allowed ranking of total seed predation from 0-6, such that samples with 'extensive' seed removal (category 2) for all three seed species was coded as 6 (e.g. 2 + 2 + 2). Since predation for each species was not independent, this pooled variable indicates relative total rates of predation per

sample. Given the spatial variability of seeds and seed removal, this was useful in identifying predation ‘hotspots’ in the field. A score of 6 could also be indicative of more thorough, voracious, or gregarious invertebrate populations or the presence of a vertebrate predator.

Invertebrate activity density was assessed using simple linear contrasts on log (Y+1) transformed data. Simple linear contrasts and Chi-squared tests were performed to compare seed predation rates and invertebrate activity density. These tests were also used to quantify differences in seed predation rates and invertebrate activity between this organic transitional study and an adjacent conventionally managed corn study (Shuler et al. in review). Caution was used not to over interpret these statistics given that these two research projects represent only a pseudo-replication.

Spatial Analysis

Geospatial analysis of invertebrate activity density and seed removal rates was conducted using Manifold System 6.50⁴ to map spatial autocorrelation onto satellite images with added GPS locations of plot boundaries and pitfall locations. Significance tests (accepted at $P < 0.01$ to reduce type I errors) were performed using Crimestat⁵, to compute Moran’s I, a standard index of covariation between different point locations. Moran’s I varies from -1 (indicating clustering) to 1 (indicating uniform dispersion). Crimestat⁵ was used to create weighted triangulated ellipses of the mean and standard deviation and applied to Manifold maps to illustrate significant spatial autocorrelation.

Results and Discussion

We presented seed predators with a free-choice assay of three weed species, making it possible that the weed species chosen and the quantity of seeds removed can give clues to which seed predator (or clade of seed predators) may have been responsible for the removal. Trends in seed predation were significantly different for each of the weed species, both by month and in terms of the likelihood of ‘moderate’ or ‘extensive’ removal ($P < 0.001$), potentially due to the variety of feeding preferences of the seed predators in our system. Laboratory feeding trials confirmed that the invertebrate taxa discussed herein would all consume seeds from at least one of the weed species offered in the field (data not shown). However, invertebrate seed preferences are challenging and little studied in the field, driven by both generalist and specialist diets (including primarily spermophagous tribes such as the Harpalini) and fundamentally limited by the maximum diameter of a seed that can be manipulated in the mandibles. Vertebrate preference for larger seeds and the tendency to consume more seeds in the same time frame as invertebrates further complicated interpretation of seed predation data. While there are certainly predators who do readily consume the weeds presented in our assay irrespective of species, we will attempt to address both pooled seed removal rates as well as trends of removal of each weed species, in order to take into account this variety of predator diet preferences.

Year was the largest source of variation in weed seed predation, accounting for 15% of total variation in removal rates ($P < 0.001$) (Figure 1.2). For all three species, seed predation was notably higher during 2006, with 1.6 times more samples showing evidence of seed predation than in 2005 ($P < 0.001$). Predation in 2005 was so scarce that nearly 50% of the sandpaper cards (153 of the 307 total) showed no predation at all, and a further 30% had only minor levels of predation. Month explained 20% of

the variation in predation in 2005, with more removal later in the season ($P < 0.001$). Given the relative lack of seed predation in 2005, further discussion of seed removal rates and correlation with fixed management factors or invertebrate activity density are presented for 2006, unless otherwise indicated.

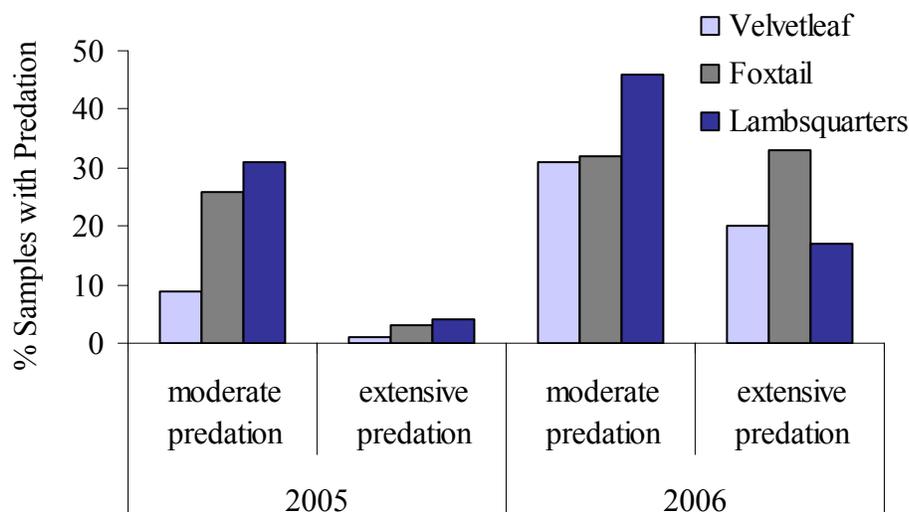


Figure 1.2. Percentage of samples with moderate, or extensive removal of velvetleaf, giant foxtail, and common lambsquarters seeds in 2005 and 2006 in transition to organic cash grains system. Moderate predation refers to 1-10 seeds removed in 48 h (1-28% removal), extensive predation to 10-40 seeds removed in 48 h (31-100% removal). For all three weed species, instances of no predation were significantly higher in 2005 ($P < 0.001$) and instances of moderate and extensive predation were significantly higher in 2006 ($P < 0.001$).

Across the two years, organic weed and nutrient management treatments had no effect on seed removal rates of the three weed species, and there was no difference between samples in the transitional organic plots and the conventionally managed control plots. These findings indicate that crop type and landscape complexity may have had a greater impact on seed predation rates than any of the weed or nutrient management treatments. Greater landscape complexity has been correlated with higher seed predation rates (Menalled et al. 1999) and it is possible that the landscape complexity of this robust experimental field study enhanced overall seed predation.

TABLE 1.1. Seed predation rates in 2006 by unrestricted access or vertebrates excluded in transitional organic grain study in Aurora, NY

Vertebrate Access by Percent of Samples Showing None, Moderate, or Extensive Predation

	No Predation ¹		Moderate Predation ²		Extensive Predation ³	
	Invert. Access Only ⁴	Vert. & Invert. Access ⁵	Invert. Access Only ⁴	Vert. & Invert. Access ⁵	Invert. Access Only ⁴	Vert. & Invert. Access ⁵
	Velvetleaf	62% ⁶	39%	20%	46%	18%
Foxtail	46%	25%	34%	30%	20%	44%
Lambsquarters	43%	32%	47%	43%	10%	24%

¹ ‘No predation’ levels were determined using controls to estimate the seed loss due to experimental error.

² ‘Moderate predation’ refers to 1-10 seeds removed in 48 hours or 1-28% removal.

³ ‘Extensive predation’ refers to 10-40 seeds removed in 48 hours or 31-100% removal.

⁴ ‘Invertebrate Access Only’ refers to seed predation assays offered under a 30 by 30 by 10 cm cap constructed from 0.5-cm wire mesh that allowed the passage of invertebrates but not birds or small mammals.

⁵ ‘Vertebrate & Invertebrate Access’ refers to seed predation assays with unrestricted access for all vertebrate and invertebrate seed predators.

⁶ Numbers in bold indicate a significantly greater percentage of samples observed within a given seed predation rate for the designated vertebrate/invertebrate access level. $P < 0.01$.

Velvetleaf predation at ‘moderate’ rates (1-28% removal) was correlated with invertebrate access ($P < 0.01$) and significantly higher in the spelt/clover mixture plots (entry A) than in the soybeans (entry B) ($P < 0.001$). Counterintuitively, vertebrates were not correlated with samples showing ‘extensive’ velvetleaf predation (31-100% removal) ($P = 0.54$) (Table 1.1). This trend is particularly surprising given that extensive velvetleaf removal measured using identical methods in the adjacent conventional corn study occurred 78% more often with vertebrate access ($P < 0.001$) (Shuler et al. in review). Though we must be cautious in comparing these two studies, it is striking that vertebrates were primarily responsible for the 45% of samples with ‘extensive’ velvetleaf predation in the nearby corn system while in the transitional

organic system located just 235 m to the NW (Figure 1.1) invertebrates were primarily responsible for the 20% of samples with ‘extensive’ velvetleaf removal.

TABLE 1.2. Invertebrate activity density over a 48-hour period in the transitional organic grain and adjacent conventional corn studies in 2005 and 2006 in Aurora NY

Species or Taxon	Invertebrate Activity Density (mean no.pitfall ⁻¹ system ⁻¹ (SE))			
	Transitional Organic Study ¹		Adjacent Conventionally Managed Study ¹	
	2005	2006	2005	2006
<i>Pterostichini</i> ⁴	● ³ 1.8 (2.5)	1.1 (1.6)	1.1 (1.5)	1.1 (1.3)
<i>Poecilus lucublandus</i>	● 3.1 (4.2)	0.9 (1.5)	●1.2 (2.5)	0.7 (1.3)
<i>Agonum muelleri</i>	● 0.8 (2.5)	0.0 (0.2)	●0.3 (0.6)	0.0 (0.0)
<i>Harpalus rufipes</i>	* ³ 1.1 (1.3)	1.0 (1.5)	0.1 (0.3)	●0.5 (0.9)
<i>H. caliginosus</i>	0.2 (0.6)	0.3 (1.0)	0.0 (0.0)	0.0 (0.0)
<i>Chlaenius nemoralis</i>	● 0.1 (0.5)	0.1 (0.2)	0.0 (0.0)	0.0 (0.0)
Other Carabids	● 0.5 (1.0)	0.1 (0.5)	0.0 (0.2)	0.0 (0.2)
Other Beetles	*2.0 (3.0)	0.4 (1.2)	● 2.8 (2.8)	0.6 (1.1)
Crickets ⁵	* 0.4 (0.90)	0.2 (0.5)	0.1 (0.4)	● 1.5 (1.7)
Other Invertebrates	0.7 (1.2)	● 2.7 (4.2)	● 1.2 (1.8)	0.7 (0.3)
Slugs ⁶	0.0 (0.0)	●0.6 (1.3)	0.0 (0.0)	● 1.6 (2.5)

¹ Study systems were analyzed separately P < 0.01.

² Between study systems, means in bold indicate the study with significantly higher activity density for each year.(P < 0.01).

³ Within each study system, means preceded by ● and * indicate the year of higher activity densities at P < 0.001 and P < 0.01 level, respectively.

⁴ Pterostichini includes *Pterostichus melanarius* Illiger, *Cyclotrachelus sodalis* LeConte, and *Abacidus (Pterostichus) permundus* Say.

⁵ Predominately Nemobiinae, occasional Gryllinae

⁶ Stylommatophora: *Deroceras* spp., *Arion* spp

The much greater activity densities of all species of carabid beetles in the transitional organic system relative to the nearby conventional corn system in 2006, may be responsible for the differences in ‘extensive’ invertebrate velvetleaf consumption (Table 1.2). The transitional organic system had higher activity densities of the Pterostichini (predominantly *Pterostichus melanarius* Illiger), *Poecilus lucublandus* Say, *Agonum muelleri* Herbst, *Harpalus rufipes* Degeer, *Harpalus caliginosus* Fab., and *Chlaenius nemoralis* Say (P < 0.01), and significantly lower activity densities for slugs (Stylommatophora: *Deroceras* spp., *Arion* spp.) and

crickets (predominately Nemobiinae, occasional Gryllinae) ($P < 0.01$). Carabid seed specialists from the genus *Harpalus* were twice-to-ten times more likely to occur in the transitional organic system (year dependant) than conventional corn system. In spite of the proximity of the two research projects, the large seed predator *Harpalus caliginosus*, was never encountered in the conventionally managed corn system.(Table 1.2).

Unlike ‘moderate’ velvetleaf removal, ‘extensive’ velvetleaf seed predation was not significantly different between spelt/clover plots and soybean plots ($P = 0.58$). This suggests that the invertebrates responsible are either (1) mobile enough to easily disperse between the 12.1 m by 36.4 m plots; or (2) are equally abundant as a feeding clade in the soybean and spelt/clover mixture plots. Given mandible sizes necessary to manipulate large velvetleaf seeds, these invertebrates are likely to include crickets, carabid beetles from the tribe Harpalini, and *Pterosticus melanarius*.

The effect of entry point on predation was the same for giant foxtail seeds as for velvetleaf, with significantly more instances of ‘moderate’ predation observed in the spelt/clover mixture than in soybeans in 2006 ($P < 0.05$) and the same lack of impact of crop on ‘extensive’ removal of giant foxtail seeds ($P = 0.43$). These entry point and vertebrate exclosure results for giant foxtail indicate that invertebrates are primarily responsible for ‘moderate’ giant foxtail removal, perhaps due to the greater year-long ground cover in the spelt/clover. Simultaneously, invertebrates moving irrespective of crop type were correlated with instances of ‘extensive’ giant foxtail predation.

Removal of common lambsquarters seeds was marginally higher for ‘moderate’ predation rates in entry point A, planted in spelt/clover ($P < 0.05$). ‘Extensive’ common lambsquarters predation was more common in entry point B, planted in soybeans, a trend that differed from that observed for giant foxtail and velvetleaf ($P < 0.01$).

Vertebrate access was correlated with a significant increase in ‘extensive’ removal of giant foxtail ($P < 0.001$) and marginally significant increase in the instances of ‘extensive’ removal of common lambsquarters ($P < 0.05$). ‘Moderate’ seed predation of both giant foxtail or common lambsquarters, however, was significantly correlated with invertebrate activity (Table 1.1).

Although invertebrate population activity density was notably higher in the transitional organic system than in the nearby conventional corn study, activity densities in the transitional organic system were inversely correlated with the increase in seed removal observed in 2006. Although there was substantially greater seed predation on all three weed species in 2006, activity of crickets and all carabid beetles, except for the large seed specialist *Harpalus caliginosus*, was significantly lower in 2006 (Table 1.2). However, spiders (predominantly Lycosidae) and other invertebrates were all captured at significantly higher rates in 2006 ($P < 0.001$). Slug capture also dramatically increased in 2006 ($P < 0.001$), although slugs are likely not responsible for ‘extensive’ removal rates given that their preference for the spelt-clover mixture plots ($P < 0.001$) and higher activity later in the season ($P < 0.05$) does not correlate with ‘extensive’ predation rates.

The inverse correlation between invertebrate seed predation and invertebrate capture rates may be explained by the lower mean temperatures and higher rainfall that occurred in 2006 relative to 2005, which can depress insect activity and lower pitfall capture rates even if abundance remains constant or increases. The view that lower activity densities in 2006 are correlated with lower temperatures, greater rainfall, or other weather-related seasonal patterns is supported by the occurrence of a similar decline in invertebrate activity densities in 2006 in the adjacent conventional corn system. If reductions in invertebrate populations in the second season of the

transitional organic system were due to greater soil disturbance or other environmental changes associated with management, we would not expect to observe the same trends in an unrelated nearby study.

Seed availability on the soil surface may also have affected seed predation rates observed. Transitioning to organic management generally leads to a 2-to-3-year pulse in weed seed production with seed production stabilizing at rates that are significantly higher than observed under conventional management (Albrecht 2005). Surface seed sampling indicated that seed availability was greater in the transitional organic system than in the nearby conventionally managed corn, potentially explain why the transitional organic system had significantly higher activity densities of invertebrate seed feeders but experienced less seed predation (data not shown). We were not able to ascertain if surface seeds were less abundant in 2006 than in 2005 in the transitional organic system, but this may have contributed to the significantly higher invertebrate seed removal rates with similar or lower activity density of invertebrates in this year.

Geospatial analysis of invertebrate populations indicated that spatial autocorrelation was common but variable for all invertebrates in both 2005 and 2006. Higher activity densities in the spelt/clover mixture plots were observed for crickets and slugs, but not for carabid species (Table 1.3). Geospatial analysis indicated that most carabids were more active in the spelt/clover plots, but uneven distributions throughout the field masked these trends. Since these uneven distributions changed with each sampling point and were consistent across the season, it is not necessary to correct for them in ordinal regression of seed predation data. This spatial autocorrelation, however, can effectively mask replicate, plot, and treatment trends on invertebrate activity density if researchers are unaware the autocorrelation is common in their system.

TABLE 3. Average invertebrate activity density over a 48-hour period in 2005 and 2006 by each entry point in Corn/Soy/Spelt-Clover Rotation

Species or Taxon	Invertebrate Activity Density (mean no. pitfall ⁻¹ system ⁻¹ (SE))			
	2005		2006	
	Entry A Soy	Entry B Corn	Entry A Spelt/Clover	Entry B Soy
<i>Pterostichini</i> ³	● 2.2 (2.9) ¹	1.5 (2.6)	0.5 (0.8)	● 1.6 (2.0)
<i>P. lucublandus</i>	● 4.0 (5.0)	●2.1 (3.1)	1.0 (1.6)	0.8 (1.3)
<i>A. muelleri</i>	● 1.1 (2.8)	0.6 (2.1)	0.0 (0.2)	0.0 (0.1)
<i>Harpalus rufipes</i>	1.2 (1.4)	1.1 (1.3)	1.0 (2.0)	0.9 (1.0)
<i>H. caliginosus</i>	0.2 (0.8)	0.1 (0.3)	0.4 (1.3)	0.2 (0.50)
<i>C. nemoralis</i>	0.2 (0.6)	0.1 (0.3)	0.1 (0.2)	0.0 (0.2)
Other Carabids	0.4 (1.1)	●0.5 (0.8)	0.1 (0.5)	0.1(0.4)
Other Beetles	1.6 (2.7)	● 2.5 (3.1)	0.5 (1.5)	0.3 (0.8)
Crickets ⁴	0.5 (1.0)	0.3 (0.6)	0.3 (0.6)	0.1 (0.4)
Spiders ⁵	0.6 (1.2)	0.8 (1.3)	●2.0 (2.0)	1.3 (1.8)
Other Invert.	0.6 (1.1)	0.7 (1.3)	● 3.2 (5.2)	1.8 (2.8)
Slugs ⁵	0.0 (0.0)	0.0 (0.0)	● 1.1 (1.7)	●0.2 (0.5)

¹ Within year, means in bold indicate the entry point/crop with significantly higher activity density.(P < 0.01).

² Between years, ● indicates year of significantly higher activity density for each entry point/crop.(P < 0.01).

³ Pterostichini includes *Pterostichus melanarius* Illiger, *Cyclotrachelus sodalis* LeConte, and *Abacidus (Pterostichus) permundus* Say.

⁴ Nemobiinae, occasional Gryllinae

⁵ Predominately Lycosidae: *Lycosa* spp.

⁶ Styломmatophora: *Deroceras* spp. and *Arion* spp.

A simple example of this phenomena is the spatially autocorrelated activity in the 2006 August sample of *Harpalus caliginosus*, a large seed specialist, whose higher abundance in entry point A, spelt/clover was occluded by clustering in the southeastern section of the field. The *H. caliginosus* population was clustered in the SE section of the organic treatment plots, predominately in the spelt, and associated with treatment 4 (Figure 1.3). There was also significant correlation between *H. caliginosus* activity density and the spelt/clover mixture of the conventionally managed control. Treatments 4 and 5 had significantly lower establishment of clover

both in the fall and by spelt harvest and significantly yields per acre of soybeans, perhaps contributing to these spatial patterns.

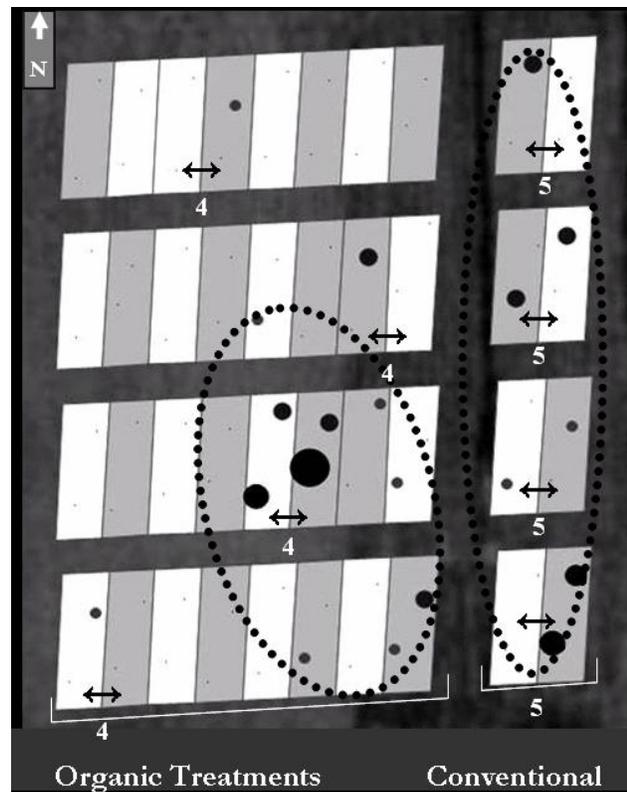


Figure 1.3. Distribution and abundance of the carabid beetle, *Harpalus caliginosus*, in spelt/clover mixture (gray) and soybean plots (white) in August 2006 in Aurora, NY. Black circles (●) indicate permanent pitfall locations and the size of circle is proportional to the number (range 1-10 individuals) of *H. caliginosus* captured in the pitfall. Dotted ellipses are the weighted triangulated mean + SD of activity densities, indicating significant spatial autocorrelation (Moran's I, $P < 0.05$). Spelt and soybean plots managed with treatments 4 (ridge tillage) and 5 (conventional control) are numbered.

Within the transitional organic system, invertebrate activity density in 2005 was almost universally higher in treatment 5, the conventionally managed control than the pooled average for the four organically managed treatments, perhaps due to the high level of disturbance from cultivation in the transitional organic plots (Table 1.4). By the second year of the transition, however, activity density of most invertebrates had stabilized and was not significantly different between the four transitional organic

treatments and treatment 5, the conventional control (Table 1.4). The Pterostichini, predominantly *Pterostichus melanarius*, and slugs were more frequently captured in organic treatment plots in 2006 ($P < 0.01$) than in the conventional control treatments while *Poecilus lucublandus* was the only invertebrate whose activity was greater in conventional control plots than organic plots ($P < 0.001$) (Table 1.4).

TABLE 1.4. Pooled invertebrate activity density over a 48-hour period for the 4 organically managed treatments (excluding the conventionally managed control trt) in 2005 and 2006, Aurora, NY.

Invertebrate Activity Density in Transitional Organic Treatments		
(mean no. cup-1 treatment-1(SE))		
Species or Taxon:	2005	2006
<i>Pterostichini</i> ⁴	1.8 (2.6)	●1.2 (1.8)
<i>Poecilus lucublandus</i>	2.6 (3.7)	0.6 (1.2)
<i>Agonum muelleri</i>	0.4 (1.0)	0.0 (0.2)
<i>Harpalus rufipes</i>	1.2 (1.4)	1.0 (1.6)
<i>H. caliginosus</i>	0.1 (0.3)	0.2 (1.0)
<i>Chlaenius nemoralis</i>	0.1 (0.5)	0.0 (0.2)
Other Carabids	0.4 (0.7)	0.1 (0.4)
Other Beetles	2.1 (3.1)	0.3 (1.2)
Crickets ⁵	0.3 (0.8)	0.2 (0.6)
Spiders ⁶	0.8 (1.2)	1.5 (1.8)
Other Invertebrates	0.6 (1.1)	2.5 (4.5)
Slugs ⁷	0.0 (0.0)	●0.8 (1.4)

¹Within year, ● indicates activity density within organic treatment block (treatments 1-4) was significantly *higher* than activity density observed in treatment 5, the conventionally managed control (means not shown) ($P < 0.01$).

²Within year, bold indicates activity density in organic treatment block (treatments 1-4) was significantly *lower* than that observed in treatment 5 the conventionally managed control (means not shown) ($P < 0.01$).

⁴Pterostichini includes *Pterostichus melanarius* Illiger, *Cyclotrachelus sodalis* LeConte, and *Abacidus (Pterostichus) permundus* Say

⁵Nemobiinae, occasional Gryllinae

⁶Predominately Lycosidae: *Lycosa* spp.

⁷Stylommatophora: *Deroceras* spp. & *Arion* spp.

To summarize, seed predation rates were significantly higher in the second year of the transition to organic cash grains. Since there was no increase in seed predation

in 2006 in the adjacent conventionally managed corn study for the three target species, this increase is likely due to higher system diversity and organic management practices than higher rainfall or lower seasonal temperatures recorded in 2006.

Instances of ‘extensive’ seed predation in the organic cash grain transition system were primarily the result of invertebrate activity, likely larger seed predators such as *Pterostichus melanarius*, *Harpalus caliginosus*, and *Harpalus rufipes*. These species were significantly more likely to occur in the organic transitional system than in an adjacent conventionally managed corn system.

Within the three crops in the transitional organic rotation, invertebrates were more active under the dense cover of the spelt/clover mixture but also dispersed into nearby soybean plots. ‘Extensive’ predation generally occurred irrespective of crop type/entry point, whether correlated with invertebrate or vertebrate activity. ‘Moderate’ seed predation of all three weed species was greater in the spelt/clover mixture and was correlated with higher invertebrate activity density in this system, particularly after correcting for carabid spatial autocorrelation.

Finally, invertebrate populations were also often significantly spatially autocorrelated, with location of clustering changing with month and species. To our knowledge, this is the first study on seed predation in agro-ecosystems that employed geospatial analysis for invertebrate activity density and associated seed removal. It is often assumed that well-designed studies are able to control for the factors influencing invertebrate populations, particularly in agricultural landscapes that – especially by contrast to unmanaged systems - are apparently uniform. These spatial analysis, however, support recent compelling research on microhabitat availability having a greater impact on invertebrate populations than the farm management or larger-scale factors typically measured in agricultural studies (Thomas et al. 2001). Simple and accessible geospatial analysis such as those presented here can be useful for

determining if unmeasured factors are driving invertebrate populations within a study system. This can be crucial given that spatial autocorrelation of an ecosystem service such as seed predation – e.g. due to clustered or asymmetrically colonizing insect populations – can easily be overlooked in (or skew) traditional statistical analyses.

Sources of Materials

¹Norton 3X Fine 150 grit, Saint-Gobain Abrasives, Inc; Worcester, MA.

²3M Brand General Purpose spray adhesive, 3M Company, St. Paul, MN.

³SPSS for Windows⁵. Release 7.0. SPSS Inc., Chicago, IL.

⁴Manifold 6.50 Enterprise Edition; CDA International Ltd.

⁵Crimestat, developed by the National Institute of Justice; Ned Levine
& Associates, Houston TX crimestat@nedlevine.com

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**2. POST-DISPERSAL WEED SEED REMOVAL BY INVERTEBRATES
AND VERTEBRATES IS VARIABLY AFFECTED BY
EXPERIMENTAL SUBSTRATE USED**

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Abstract

A standardized methodology for evaluating seed predation by invertebrates is needed to increase our capacity to generalize across studies. This work can increase our ability to transition beyond simple ecosystem services assessments into providing a practical set of recommendations for agricultural managers that enhances invertebrate weed seed predation. The objective of this study was to compare the effect on seed predation levels of two common experimental methods of offering weed seeds to predators: the sandpaper and soil substrate methods. Invertebrate seed predators and associated weed seed predation levels were assessed in July, August, and September of 2005 and 2006 within a conventionally managed corn system. Seed predation levels of three common weed species: velvetleaf, giant foxtail, and common lambsquarters were estimated with feeding trials (40 seeds of each species offered over a 48-h period) using these two methods. As a corollary, we investigated the use of geospatial analysis to estimate spatial autocorrelation of invertebrate populations and seed removal rates. Results suggest caution in using synthetic substrates, such as sandpaper, when assessing predation of small-seeded species ($>1 \text{ mg seed}^{-1}$), or when seed predators are predominantly invertebrates. By contrast, predation of the larger-seeded species velvetleaf and giant foxtail were unaffected by sampling method, perhaps due to greater removal by vertebrates. One possible solution for overcoming concerns about the sandpaper sampling would be to use it only for larger seeds. Studies necessitating the use of small seeded species could include a small number of soil substrate samples to calibrate the impact of the sandpaper method on seed predators. Our geospatial evaluation revealed significant spatial autocorrelation by insect species and by sampling month, with invertebrate seed removal showing associated clustering. Without investigating this spatial component we would not

have observed the strong positive correlation between *P. lucublandus* activity and giant foxtail and lambsquarters predation by invertebrates. Failure to consider the effect of clustering in insect populations and associated seed removal rates can cause important effects and/or trends to be masked when averaged across an entire system.

Nomenclature: Cry3Bb Bt; tefluthrin; *Abutilon theophrasti* Medicus # ABUTH; Common lambsquarters, *Chenopodium album* L. # CHEAL; velvetleaf, Giant foxtail, *Setaria faberi* Herrm. SETFA; corn, *Zea mays* L.

Key words: Biological control, Bt-corn, Carabidae, ground beetle, genetically-modified crops, GIS, geospatial analysis, methodology, seed bank dynamics, seed predators, seed predation, spatial autocorrelation.

Introduction

The vast majority of seed predation studies have focused on relatively intact forests and prairies (Janzen 1971). In the last decade, however, attention has turned toward investigating seed predation in anthropogenic environments, particularly agro-ecosystems, because changes in weed seedbanks can be both ecologically and economically important (Menalled et al. 2000; Westerman et al. 2003; Gallandt et al. 2005; O'Rourke et al. 2005). Controlling populations of annual weeds is one of the major challenges in current agricultural production. The deleterious effects of weeds are estimated to cost the U.S. economy more than \$15 billion annually, more than the control costs of diseases and insect pests combined (Bridges 1994). The current paradigm for managing these weeds relies heavily on the use of herbicides (Jordan 1996).

Generalist invertebrates that feed on weed seeds may be one of the most important broad-spectrum natural forms of biological weed control impacting weed population dynamics in natural and agronomic systems (Crawley 2000; Cromar et al. 1999). This effect on weed populations results in lower seed abundances (Gonzales-Andujar and Fernandez-Quintanilla 1991; Jordan et al. 1995; Davis and Liebman 2003; Davis et al. 2003; Liebman et al. 2003; Landis 2005) and changes in weed community composition (Carroll and Risch, 1984; Tooley and Brust 2002). While seed removal via predation may not entirely replace cultivation, herbicides or other weed management practices, it can be important within organic or integrated weed management systems .

It is difficult to quantify levels of post-dispersal seed predation, much less what portion of that predation can be attributed to insects versus vertebrates. Both within and across studies, the taxa of principal seed predators have been variable, with

vertebrates showing a tendency to be more consistent in predation rates over the growing season where they are present and invertebrates exhibiting more variable rates, perhaps related to species-specific phenology (Marino et al. 1997; Westerman et al. 2003). The relative importance of invertebrates and vertebrates as weed seed predators has resurfaced in recent years as multiple studies corroborate the fact that invertebrates can be the dominant predators of weed seeds in agricultural systems (Brust and House 1998; Cromar et al. 1999; Honek et al. 2003; Gallandt et al. 2005). Currently, research is focused on understanding which agricultural systems and farm management practices are most hospitable to beneficial invertebrates, and can lead to seed predation levels that have a significant negative impact on weed communities.

The difficulty of generalizing results from the numerous published seed predation studies into a coherent meta-analysis is what drives the controversy surrounding the relative importance of invertebrates and vertebrates as seed predators in agro-ecosystems and the types of management that may enhance seed predation. In large part, this difficulty is due to the complexity and vast array of agro-ecological environments in which the studies are performed. Seed predation research to date has varied so much in terms of geographic location, crop species and rotations used, and management practices employed that it is difficult to pool inferences across studies. Thus far, this work has shown that the relative abundance and impact of the distinct seed predator guilds vary among habitats (Hulme 1998; Menalled et al. 2000), but can be affected by crop management tactics used (Carmona and Landis 1999; Gallandt et al. 2005).

This study addresses the second major source of variation between seed predation studies: namely, the variety of methods used to assess seed removal. Choice of experimental seed predation substrate and methodology is justifiably based on convenience, given that the research focuses on comparisons of treatments or other

factors that assume no treatment by method interaction. The five most commonly used sampling methods are distinguished from one another based on whether they use (1) natural or synthesized substrate, (2) adhesive material such as glue or tape to temporarily hold seeds to the substrate, and (3) the relative difficulty of processing samples (Table 1.1). The two methods that were selected for further study were: (a) the sandpaper substrate, and (b) soil substrate methods. The soil substrate method was chosen because it is the closest approximation to natural field conditions. While it requires extensive field installation time and several hours of laboratory processing per sample to recover seeds, it is the most useful estimate of absolute seed predation rates that may minimally impact predator behavior. The sandpaper substrate method was selected as the best representative of the time and cost effective seed predation techniques and since its surface when covered with soil appears most similar to a natural field topology.

The most comprehensive investigation to date of the impact of experimental substrate on seed removal rates is a small study published as a note by Gallandt (2005). This study summarizes well the diversity of seed predation assays currently in use and compares different experimental substrates in the field to determine methodological effects on seed predation. Performed in a two-hectare red clover (*Trifolium pratense* L.) field near Stillwater, ME, seeds of six common weed species were offered using 3-6 different predation methods (depending on year) over a three-day period in July.

Predation rates in this study were not consistent across all methods. Invertebrate predation which was estimated using vertebrate exclosures, in particular showed variation potentially related to the different apparency of weed species on different media. Overall, seeds were more difficult for invertebrate predators to find against a background of soil (whether using the soil method or in methods where soil is dusted

on top of media) relative to other substrates. Predation rates by method were also highly dependant on seed type, with some species being more responsive to methodological differences. These clear differences in weed species by method interactions even in a small-scale study indicate that more comprehensive research is needed to determine the most suitable methods for assessing seed predation.

Table 2.1: Overview of the the five most commonly used seed predation sampling methods focusing on setup/processing time and approximation of the substrate to resemble field conditions.

	Pad Method	Tape Method	Sandpaper Method¹	Sand Method	Soil Method²
Setup	scouring pad topped with seeds	seeds on card with double sided tape, soil covered	aerosol glue holds seeds + sandpaper, soil covered	seeds placed on sand in dish flush w/ ground	seeds placed on field soil in dish flush w/ ground
Time	very fast no lab setup	relatively fast in lab setup & processing time	setup & processing rel. fast	slower setup, variable processing	slower setup, very long processing
Pros	easy setup & processing, reusable	closer to field surface than pads	less synth. no adhesive impact	natural, less invertebrate avoidance	natural, minimal invert. impact
Cons	all synthetic small seeds lost in pad	tape has scent & can trap inverts.	small seeds adhere too tightly	sand causes increased seed searching	processing time nearly prohibitive
	all synthetic	synth. +soil	synth. + soil	all sand	all soil
	none	tape	aerosol glue	none	none
	→ → → →	slower processing time			→ → → →
	→ → → →	substrate closer to field conditions			→ → → →

¹ We chose to compare the soil and sandpaper methods in this study. The soil method acts as an invertebrate behavioral control treatment because it closely approximates field conditions.

² Sandpaper method was chosen as a contrast to the soil method because it is the most time and cost effective seed predation technique with minimal impact on invertebrate behavior.

Concerns about the effect of specific sampling methods on animal behavior in seed predation studies may be less important when seeds of multiple species are offered

(Brust and House 1988; Cromar et al. 1999; Menalled et al. 2000; Westerman et al. 2003). However, recent work has demonstrated that predator behavior can be substantially affected by the way in which seeds are presented (see Gallandt 2005; O'Rourke et al. 2005). This impact on behavior suggests that published predation rates in agro-ecosystems may be over- or under-estimating the seed removal capabilities of taxa that are drawn to or avoid certain experimental sampling setups.

In an effort to increase our ability to generalize across studies, research focused on standardizing seed predation assays is needed. There is also legitimate interest in how different media and methods for offering weed seeds affects seed predator behavior in the field (Gallandt 2005; O'Rourke et al. 2005). A standardized seed predation methodology is essential for the development of this area of research. This work is also critical given that seed predation research currently suffers from a frequent inability to transition from an ecosystem services assessment to providing a practical set of management recommendations to agricultural managers. The objective of this two-year field study was to assess the relative impacts of offering seeds of three common annual weeds on a (1) sandpaper substrate, and (2) soil substrate on predator behavior and seed removal levels.

As a corollary, we also investigated the use of geospatial analysis to estimate spatial autocorrelation of invertebrate populations and seed removal rates. To our knowledge, no other research on seed predation in agro-ecosystems has employed geospatial analysis of invertebrate activity density and associated seed removal. We hypothesized that spatially autocorrelated seed removal rates would be observed due to clustering of invertebrate seed predator populations. Spatial clustering (or skewness) can easily be overlooked using traditional statistical analysis, adding to the difficulty of generalizing findings across different studies.

Materials and Methods

Location and Species Tested

Samples were collected at the Robert B. Musgrave Research Farm of Cornell University near Aurora, NY (42°45' N, 76°35' W). Soil is a subsurface-drained Kendaia-Lima silt loam soil (fine-loamy, mixed, nonacid, mesic Aeric Epiaquept-Oxyaquic Hapludalf). Six replicate plots of 51 by 46 m were planted to corn and subjected to several treatments in a randomized complete block design. Seed predation methodological comparisons were conducted as part of a larger investigation of corn rootworm *Diabrotica* spp. (Coleoptera: Chrysomelidae) control techniques. Treatments consisted of: a Cry3Bb Bt hybrid, Monsanto Yieldguard[®] Rootworm control (MON863); a non-Bt isolate treated with the broad spectrum insecticide tefluthrin¹ applied at planting at a rate of 388 mL per 1000 m row); and a control consisting of the non-Bt isolate with no corn rootworm control. Treatments are referred to as Bt, tefluthrin, and control, respectively. All seeds were treated with the fungicide N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide (Captan)² at the label rate of 2.3 ml kg⁻¹ of seed). A minimum of 6-12 corn rows buffered plots from one another and the edges of the field to limit edge effects. The field was managed as described above for two years prior to the start of the study.

Given that Bt endotoxin has a greater effect on the larval stage of beetles, the Bt produced in the first year would have a proportionally larger impact on the larvae that hatch and mature in the field during the first growing season or winter (depending on a species reproductive phenology). Adult activity densities of carabids and other beetles would therefore likely not be affected by Bt toxins until the second season when the mortality in the larval population would impact the adult population. Insecticides, by contrast, have a significantly deleterious effect on both adult and larval populations in

the first year (Lesiewicz et al. 1984; Brust et al 1986; Reed et al. 1992; Chen and Willson 1996). Thus initiating the seed predation study after several years of management helps to mitigate the differences between the effects of insecticides and Cry3Bb Bt toxins on adult carabid activity densities and, therefore, on any associated weed seed predation.

Seed predation rates were determined for three annual weeds, common lambsquarters (*Chenopodium album* L.), giant foxtail (*Setaria faberi* Herrm.), and velvetleaf (*Abutilon theophrasti* Medic.). These are three of the most common and troublesome agricultural weeds in New York (Bridges 1992; Hartzler et al. 1993; Singer et al. 2000). Seeds were collected on site at the Robert Musgrave Research Farm in 2003 and 2004 and stored in paper bags at 3.5 C to inhibit germination and enhance long-term viability. The wide range in average seed size of these three species (7.3 mg for velvetleaf, 1.6 mg for giant foxtail, and 0.70 mg for common lambsquarters) was helpful for attracting different weed seed predators as well as for determining possible interaction effects between seed size and sampling method.

The seed density of each weed species offered on either the sandpaper or soil substrate was 1000 seeds m⁻², with a combined seed density of 3000 seeds m⁻², levels typical of temperate cropping systems with moderate weed seed production. The suitability of the seed density used was confirmed by on site sampling of the surface-available seedbank (data not shown). This total seed density was comparable to or less than that used in similar experiments (Brust and House 1988; Cardina et al. 1996; Cromar et al. 1999), and far lower than the combined density of 25,211 seeds m⁻² used by Gallandt (2005). Since seed predation may be density dependent, using a seed density typical of our system may reduce density-dependent inflation of predation rates (Cardina et al. 1996; Cromar et al. 1999).

Invertebrate Pitfalls

Within each of the fifteen plots, locations for eight pitfalls were selected randomly. Pitfall traps consisted of a buried plastic cup (9 by 12 cm) with the upper rim flush with ground level, and smaller inner cup (7.5 by 4.5 cm) serving as a lining for easy specimen removal. Eight pitfall traps per plot were opened for a 24-h period every week, from planting to harvest (May-to-September). Arthropods were identified and transferred to jars containing 70% ETOH. Once monthly, and depending on weather, a randomly selected six of the eight pitfalls per plot were assigned seed predation sampling setups. These pitfalls were filled with ethylene glycol and kept open for a 48-h period. After this period, arthropods were identified, washed in water to remove the ethylene glycol, and transferred to vials filled with 70% ETOH.

Evaluation of Seed Predation Levels

Sampling was conducted once monthly from June-to-September during a 48-h window beginning between 9A.M. and 11 A.M. Eastern Daylight Time (EDT). A randomly selected six of the eight pitfalls were assigned either sandpaper or soil substrate setups; with two total soil and four total sandpaper setups per plot. This unbalanced design with fewer soil method samples and more sandpaper method samples per plot was used because of the reduced processing time needed using this combination of sampling methods, thus increasing total sample periods per season. To avoid seed loss during rainfall events, sampling was carried out when the weather forecast called for less than 20% probability of rain. Samples were discarded if any precipitation occurred during the 48-h window. These precipitation restrictions ultimately resulted in a total of three sampling periods in each year (i.e. June/July, August, and September).

To quantify the portion of target seeds removed that could be attributed to vertebrates rather than ground-dwelling arthropods, vertebrate exclosures were fitted on half the seed predation assays established during each sampling period. The vertebrate exclosures consisted of a 30 by 30 by 10 cm cap constructed from 0.5-cm wire mesh allowing the passage of beetles and other invertebrates but not small mammals. In preliminary work, no differences in foraging behavior were observed for invertebrates and vertebrates in the presence of these caged exclosures (data not shown).

To calculate experimental error associated with this sampling method, 1.5- by 1.5-mm window screen was affixed over the vertebrate exclosure to deny all predators entry. At each sampling period, total exclosures were placed over ten sandpaper predation substrate setups and ten soil substrate predation setups and processed to determine the percentage seed loss associated with sampling error rather than predation. This was particularly important to determine for the soil substrate method because the recovery of seeds using this method is a complicated, multi-step process involving several sample transfers, elutriation, and often, different experimenters.

Soil Substrate Method

Soil was collected from the Mt. Pleasant Research Farm Facility of Cornell University, 10 km east of Ithaca, NY and was a Mardin silt loam that did not contain seeds of the three target weed species nor other common agricultural weeds. Soil was offered in arenas buried flush with the ground consisting of a 20 by 20 cm PVC frame 2 cm deep. The bottom of the arena consisted of hot glued 0.5- by 0.5-mm nylon window screen, providing a moisture permeability that enhanced surface uniformity with surrounding soil after morning dew and decreased the likelihood of velvetleaf germinating during the experimental window. Care was taken to ensure a continuous

substrate surface between the arenas and the surrounding field. Soil in arenas was left in the field for 48 h and then carefully removed and placed in bags sewn from 0.5- by 0.5-mm nylon window screen (breathable fabric again inhibited germination of seeds, particularly velvetleaf). Bags were immediately transported to the laboratory and dried at 40 C for at least 24 h. This procedure insured that none of the target weed seeds germinated in the bags. Weed seeds present in the dried soil were extracted using a high volume hydraulic elutriator, dried, sorted, and counted to determine the percentage of seeds removed.

Sandpaper Substrate Method

Sandpaper with maroon/brown backing³ was cut in half from its standard 23 by 28 cm to 23 by 14 cm formats. Adhesive⁴ was sprayed lightly and evenly over the surface of the card for 2 s at a distance of approximately 30 cm. Forty seeds each of the three target weed species were placed evenly over the sandpaper surface, making sure to avoid placing seeds on perimeter edges. A small glass jar was gently rolled over the card, to increase surface area contact of the larger velvetleaf seeds with the adhesive. A fine layer of seed-free soil was then dusted over the entire card surface using a large chef's canister with a perforated lid designed for dusting spices. After 30 min., the adhesive was dry and cards were carefully placed in plastic bags. This protocol is a slightly modified version of the procedure used by Westerman et al. (2003) and O'Rourke et al., (2005). After sampling, the sandpaper cards were bagged and depending on the size of weed species used, seeds were sieved and counted.

Data Analysis

Seed predation rates were coded as dummy variables for PLUM ordinal regression with a negative log-log or probit link function (depending on dependant

variable distribution) using SPSS for Windows. The PLUM ordinal regression procedure in SPSS also produced both Cox and Snell and Nagelkerke pseudo R-Square measures to estimate percent of variation in the dependant variable explained by the model. The CROSSTABS procedure in SPSS was used to perform contingency table analysis on ordinal seed removal rates and seed predation method, vertebrate exclosure, year, month of sampling, corn treatment, and plot location in the field. CROSSTABS produced Chi-square tests and directional Somers'd, a measure of association between ordinal variables with a range from -1 to 1 indicating the intensity of correlation (1 indicating perfect positive correlation). Statistical significance was accepted at the 0.01 alpha level to reduce the possibility of type I error from multiple testing.

Ordinal categories for seed removal of each weed species were coded as, 0 = no predation, 1 = moderate seed predation (1-10 seeds removed or 1-28% removal), 2 = extensive seed predation (10+ seeds removed or 31-100% removal). These categories were determined after assessing experimental error associated with each method, as indicated by the control treatment that denied all predators access to each seed predation setup. Seed loss due to processing and other sources of experimental error varied by method and by size of weed species. The range across methods and seed types included an error of +/- 1 seed for large-seeded velvetleaf recovered from the sandpaper method to an error of +/- 3 seeds associated with the small-seeded common lambsquarters from the soil method.

A final dependant variable was constructed by adding the ordinal variables from all three weed species into one column. This allowed ranking of total seed predation from 0-6, such that samples with "extensive" seed removal (category 2) for all three seed species were coded as 6 (e.g. 2+2+2) and those samples, for example, with extensive seed predation on only one species and no seed predation on the other two

were ranked as 2 (e.g. 2+0+0). Since predation on each species was not independent, this pooled variable indicates relative total rates of predation per sample. Given the spatial variability of seeds and seed removal, this was useful in identifying predation ‘hotspots’ in the field. A score of 6 could also be indicative of more thorough, voracious, or gregarious invertebrate populations or the presence of a vertebrate predator.

Spatial Analysis

Geospatial analysis of invertebrate activity density and seed removal rates was conducted using Manifold System 6.50⁶ to map spatial autocorrelation onto satellite images with added GPS locations of plot boundaries and pitfall locations. Significance tests (accepted at $P < 0.01$ to reduce type I errors) were conducted using Crimestat⁷, to compute Moran’s I, a standard index of covariation between different point locations that produces a correlation coefficient varying from -1 (indicating clustering) to 1 (indicating dispersion). We used z values of predation or invertebrate counts applied over x and y values of the latitude and longitude of pitfall locations to test for significance. Crimestat was also used to create weighted triangulated ellipses of the mean and standard deviation (Figure 1.3) applied to Manifold maps in order to illustrate significant spatial autocorrelation.

Results and Discussion

Seed removal rates varied by weed species, but 83% of all seed predation samples over the two years and six total months had moderate seed predation (1-28% removed) on at least one weed species. Velvetleaf predation was highest: 47% of all pitfalls showed extensive predation (31-100% seeds removed) and a further 15% of the pitfalls had at least moderate predation (1-28% removed) (Figure 2.1). Foxtail removal rates were 30.3 % for moderate predation and 21.2% for extensive rates

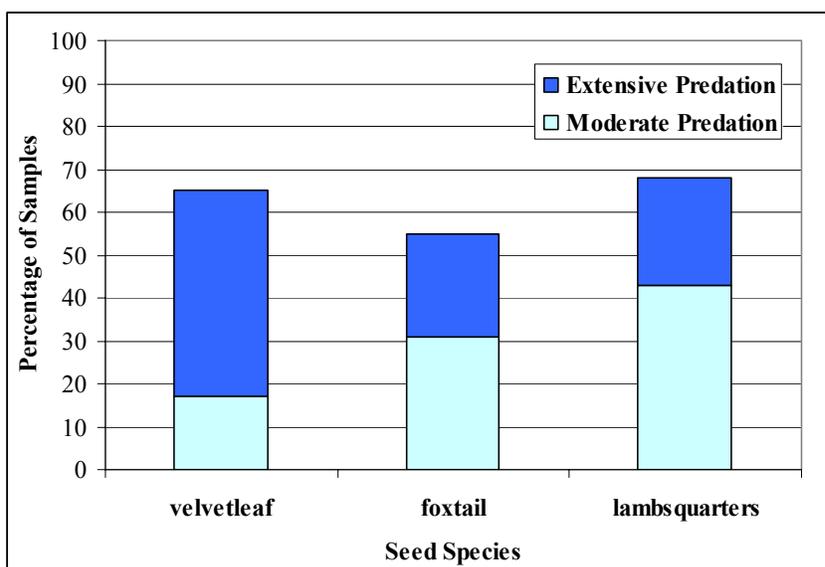


Figure 2.1. Percent of samples from 2005 and 2006 showing velvetleaf, foxtail, and lambsquarters predation. Moderate predation refers to 1-10 seeds removed in 48 hours or 1-28% removal, extensive predation is 10-40 seeds removed in 48 hours or 31-100% removal.

(Figure 1). Common lambsquarters rates of moderate and extensive predation were 41.5% and 19.7 %, respectively (Figure 2.1). Across all weed species, both year of sampling and treatment (Bt, tefluthrin and control) had no significant impact on predation.

Significantly more seed predation occurred using the soil substrate method than the sandpaper substrate method ($P < 0.001$) (Figure 2.1). Instances of extensive seed removal of all three species (an ordinal value of 6) was observed three times as often on soil substrates than on sandpaper cards. Considering the proportion of predation attributed to invertebrates alone, significantly more soil samples than sandpaper samples had observed values of 2-6, and significantly fewer soil substrate samples suffered the two lowest predation classes (0,1) ($P < 0.001$).

Seed predation method explained 10% of the variation in pooled total removal rates, and 8% of the variation in pooled total removal attributed to invertebrates only ($P < 0.001$). However, using ordinal regression of each weed species separately, between 16% and 18% of the variability in common lambsquarters removal was explained by sampling method ($P < 0.001$), which dropped only slightly to 12% when considering removal attributed to invertebrates alone ($P < 0.001$). The large impact of method on common lambsquarters seed removal levels may be due to its predators being primarily invertebrates. There was no significant difference between common lambsquarters removal with and without the vertebrate exclosures and no more of the variation in removal was explained by adding the exclosure variable to the ordinal regression model. Smaller seeds are more difficult to locate and offer fewer resources per seed, making them less desirable targets for generalist vertebrates and more likely to be predated upon primarily by invertebrates, especially predispersal specialists (Hulme 1998).

These results indicate that the sandpaper method impacts insect behavior, particularly predators of the small-seeded common lambsquarters, either through avoidance of the substrate or altered seed searching performance. The only significant predictors of common lambsquarters predation were method used (Figure 2.2) and sampling month, with greater predation earlier in the season. Inclusion of invertebrate

counts as covariates resulted in no significant association between any insect species and seed removal levels; however, slugs (*Stylommatophora*: *Deroceras* spp., *Arion* spp.) were positively correlated with higher seed predation levels. While slugs are known seed predators, slug populations peaked during the wet spring and early summer and declined during the season. Thus slugs may be important seed predators of common lambsquarters, but their seasonal variation may be acting as a proxy for greater weed consumption by invertebrates early in the season due to lower total abundance of seeds. Given their sensitivity to microsites, slug numbers may also be indicative of a moist microsite amenable to other invertebrate seed predators that are less abundant in the field or more spatially autocorrelated.

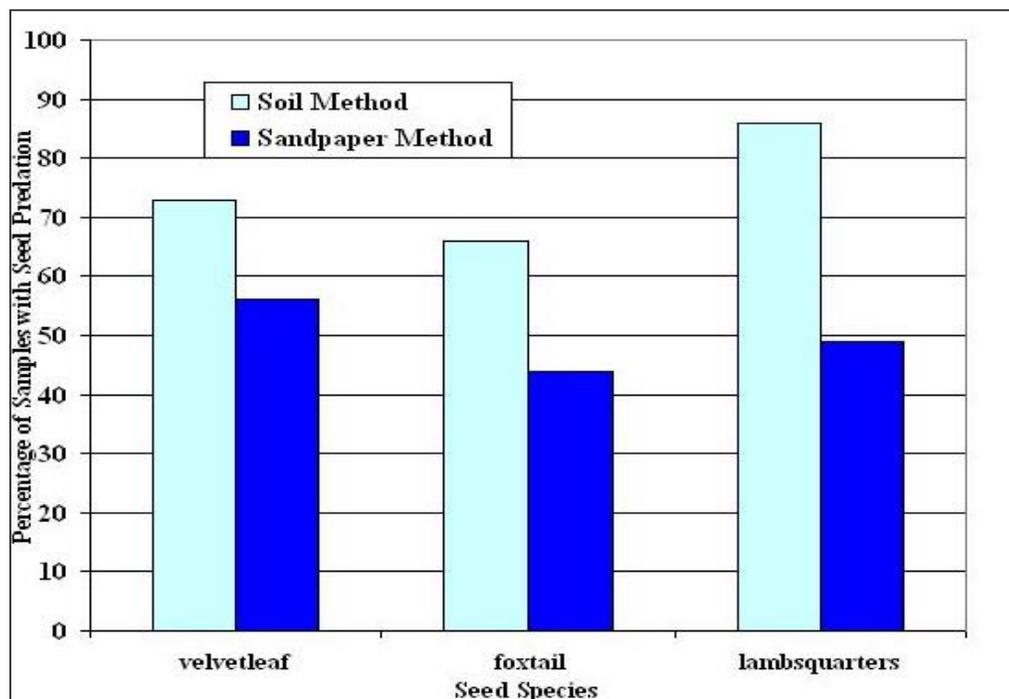


Figure 2.2. Percent of samples showing by seed predation method showing removal of velvetleaf, foxtail, and lambsquarters in 2005 and 2006 Starred bar (*) indicates significantly more instances of predation using this

By contrast to common lambsquarters, only 2% of the variation in velvetleaf seed removal by all predators was explained by the sampling method used ($P < 0.001$).

Similarly, just 2% of variation in removal attributed to invertebrates alone was explained by sampling method, but this model was only marginally significant when controlling for type I errors ($P < 0.05$). Presence of the vertebrate enclosure decreased odds of extensive velvetleaf predation (vs. moderate or no predation) by a factor of 0.29 ($P < 0.001$). Extensive velvetleaf removal occurred 78% more often with vertebrate access and instances of no predation were almost twice as likely with the vertebrate enclosures in place. A total of 9% of the variation in velvetleaf seed removal could be attributed to the presence or absence of vertebrate enclosures ($P < 0.001$). These results indicate that vertebrates are primarily responsible for significant velvetleaf predation (versus none at all) increasing by a factor of 2.9 when using the soil rather than the sandpaper sampling method. While vertebrates were primarily responsible for velvetleaf predation, pitfall counts of the carabid beetle *Pterostichus melanarius* Illiger were positively correlated with higher removal of velvetleaf that was attributed to invertebrates only ($P < 0.001$). *P. melanarius* is an introduced common ground beetle known for being an important predator of insects and plants in annual cropping systems (Thomas et al. 1998)

Unlike the early season removal associated with common lambsquarters, velvetleaf seed predation rates increased steadily during the growing season. Each month later in the year increased the likelihood of significant velvetleaf predation by 2.3 times with month explaining 9% of the variation in velvetleaf removal ($P < 0.001$). Distributions of removal of velvetleaf seeds from the 0-2 category followed an inverted bell curve, with 203 instances of no predation decreasing to 79 instances of moderate predation, and increasing again to 250 instances of extensive predation. This ‘all-or-nothing’ approach may be indicative of larger predators (e.g. mice and birds) that can disperse or consume 15 or more seeds in one location. This distribution, combined with the impact of enclosures on removal rates, indicates that the most consistent

predators of velvetleaf are likely vertebrates. Given the impact of vertebrates on this species, the observed month effect may be due to the higher weed seed rain later in the growing season that creates abundant resources known to attract larger vertebrate populations and drive up seed predation rates (Westerman 2002).

The impact of sampling method used on giant foxtail was similar to the observed effect on velvetleaf seed predation (Figure 2). While 6% of the variation in removal of giant foxtail by all predators was explained by sampling method used ($P < 0.001$), the percentage variation in seed removal attributed to invertebrates alone explained by method used dropped to 2% ($P < 0.05$). Contingency table analysis of method used and invertebrate giant foxtail removal confirmed the marginal significance of the ordinal regression model. This lack of significance indicates that predators of giant foxtail in our system are relatively unaffected by sampling method used, though the overall lower rates of extensive giant foxtail predation may also be directing this trend. Vertebrate access was correlated with a greater likelihood of extensive giant foxtail seed removal, explaining 4% of the variation in removal rates ($P < 0.001$). Invertebrates appear to be responsible for instances of moderate giant foxtail removal, since vertebrate access explained only 1% of the variation in moderate removal and was only marginally significant ($P < 0.05$). Month had a significant though not very large impact on removal, with slightly more giant foxtail predation later in the season ($P < 0.001$).

There was also significant spatial variation in giant foxtail and common lambsquarters predation. Using a simple spatial variable that scored plots 1-15 from the northeast to the southwest, plots 1-5, which make up the eastern portion of the field were determined to have significantly higher giant foxtail and common lambsquarters predation levels ($P < 0.05$). For giant foxtail, adding this spatial component alongside the independent variables of predation sampling method and

vertebrate exclosure increased the pseudo R^2 value to 15% from 9% (model $P < 0.001$). For each one unit increase in plot moving from the northeast to the southwest, the odds of moderate or extensive predation on giant foxtail or common lambsquarters decreased by a factor of 0.92 ($P < 0.01$).

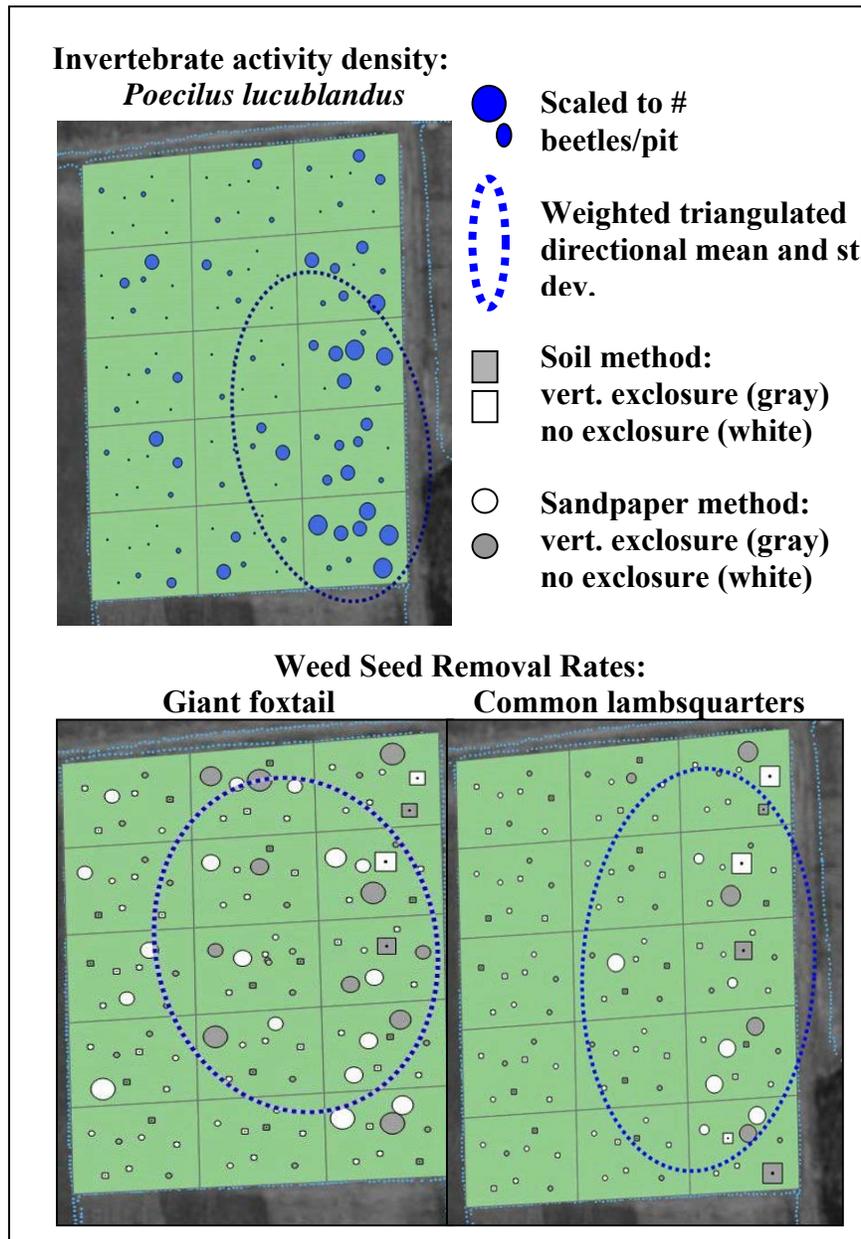


Figure 2.3. Pooled August 2005 and 2006 data for activity density of *Poecilus lucublandus* (left) and removal of giant foxtail and common lambsquarters (right). Ellipses denote significant spatial autocorrelation (Moran's I, $P < 0.01$).

This spatial variability may be due to the eastern third of the field draining less rapidly after rain events and remaining slightly moister even in dry months (R.E. Shuler, personal observation). It is possible that these very slight moisture differences were enough to drive population abundances of important giant foxtail predators. Given that both moderate and extensive predation rates are impacted for both giant foxtail and common lambsquarters, this spatial environmental component must be either (1) affecting both vertebrates and invertebrates identically; or (2) affecting key invertebrate seed predators enough to increase both moderate and extensive predation of giant foxtail and common lambsquarters in the eastern section of the field.

Geospatial analysis of seed predation using Manifold spatial software to visualize spatial autocorrelation of common lambsquarters and velvetleaf removal confirmed the clustering in the east obtained from the binary logistic regression. Moran's I values, measures of spatial autocorrelation, were significant in August samples for both common lambsquarters and giant foxtail removal ($P < 0.01$) (Figure 2.3). Activity density for the important seed predator *Poecilus lucublandus* Say was also significantly spatially autocorrelated and located in the same eastern portion of the field (Figure 2.3).

There are three important implications from the findings reported herein. First, researchers should be particularly cautious in using synthetic substrates when assessing predation of small seeded species, especially those less than 1 mg seed^{-1} , or when seed predators are predominantly invertebrates. This was illustrated in this study by the larger impact of sampling method used on common lambsquarters, the species with the smallest seed in the study (0.70 mg), and the only species whose seeds were predominantly removed by invertebrates.

Second, predation rates of larger seeds such as velvetleaf or giant foxtail appear to be attracting different predator guilds than smaller-seeded common lambsquarters and

were less impacted by the sampling method used in our system. Data from the vertebrate exclosures therefore suggest that these differences may be due to vertebrates being the most consistent seed predators of the larger-seeded weed species used. In a study primarily interested in vertebrate predation, the sandpaper substrate sampling method would therefore be an adequate and expedient procedure for assessing velvetleaf and giant foxtail seed removal. Giant foxtail seed removal by invertebrates in particular showed no correlation with method used, although this may have been partially affected by the lower levels of 'extensive' giant foxtail removal in our study.

Third, geospatial analysis of seed predator activity density can help reveal clustering of insect populations that can otherwise skew statistical analysis. In this study, spatial autocorrelation explained between 6% and 9% of the variation in giant foxtail and common lambsquarters removal rates attributed to invertebrates alone. Similar clustering of large populations of *Poecilus lucublandus* suggests that this carabid may be driving invertebrate removal of these two weed species. Without investigating the spatial component, the impact of *P. lucublandus* on invertebrate giant foxtail and common lambsquarters predation would have been masked as it was averaged across the entire system. Researchers should therefore be careful not to neglect the impact of clustered insect populations and associated seed removal rates.

One particularly parsimonious solution for addressing the impact of sampling method on seed removal would be to include a small number of soil substrate samples in such studies to evaluate the site and species-specific impact of the sandpaper sampling method on predator taxa and seed removal levels. Once this relationship has been calibrated for a given study system, researchers can then proceed with sampling using only the rapid sandpaper method. This early assessment of the impact of

sampling method on predator behavior and seed removal levels could then be considered during data analysis.

Sources of Materials

¹ Force 3G[®], Zeneca Agrochemicals, Syngenta Corporation; Wilmington, DE.

² Captan, Drexel Chemical Co., Memphis, TN.

³ Norton 3X Fine 150 grit, Saint-Gobain Abrasives, Inc; Worcester, MA.

⁴ 3M Brand General Purpose spray adhesive, 3M Company, St. Paul, MN.

⁵ SPSS for Windows5. Release 7.0. SPSS Inc., Chicago, IL.

⁶ Manifold 6.50 Enterprise Edition; CDA International Ltd.

⁷ Crimestat, developed by the National Institute of Justice; Ned Levine & Associates, Houston TX crimestat@nedlevine.com

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