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Undercover predators: Vegetation mediates foraging, trophic cascades, and biological control by omnivorous weed seed predators

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UNDERCOVER PREDATORS: VEGETATION MEDIATES FORAGING, TROPHIC

CASCADES, AND BIOLOGICAL CONTROL BY OMNIVOROUS WEED SEED

PREDATORS

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To Philip Keeney, Rosie Kaplan and Grandma Midge

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ABSTRACT

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Weed pressure is the most costly challenge that vegetable growers face, requiring more labor investment than other production inputs. Vertebrate and invertebrate seed predators destroy a large percentage of weed propagules on the soil surface, and their ecosystem services may ease labor requirements for farmers in herbicide-free systems. Cover provided by living vegetation is an important predictor of seed predator activity, and my dissertation takes a comprehensive approach to understanding the behavior, predator, and environment-mediated mechanisms by which cover impacts weed seed predation in crop environments.

First, I performed a meta-analysis of 27 studies to quantitatively evaluate what is currently known about seed predation by vertebrates and invertebrates across weed species, crop environments, and seasons (Chapter 1). I found that that seed predators impact some weed species more than others, depending on taxa-specific seed preferences, and that predation rates are minimal in environments entirely void of vegetation. Next, in Chapter 2, I examined the role of vegetative over in determining oviposition preferences of *Harpalus pensylvanicus,* the most common carabid seed predator in Midwestern crop systems. I found that while adult beetles were strong dispersers and foraged in a variety of habitat types, larvae were less mobile and more vulnerable to disturbance than adults. They were almost exclusively captured in environments characterized by long

disturbance intervals and abundant living biomass, emphasizing the importance of cover as perennial refuge for maintaining stable populations of natural enemies.

In Chapter 3, I examined omnivorous predator assembly around basic biological resources (cover, seeds, and prey). I found that both predaceous and omnivorous carabid species aggregated in patches of vegetative cover and omnivores assembled in seed patches. None, however responded to prey availability. Using food-specific protein markers, I found that cover doubled the likelihood of detecting seed material, but not prey material in beetles' digestive tracts. This implies that omnivorous carabids are competent biological control agents of weed seeds, and that provisions of plant cover will not only attract more seed predators, but also induce their seed-feeding behavior. Even though cover directly facilitates seed-feeding, it may also increase the likelihood of intraguild predation on invertebrates by small mammals, as both taxa utilize the same refuge environments. In chapter 4, I quantified the cascading effects of behavior mediated predator-prey interactions over four trophic levels. I found that use of cover by small mammals avoiding predation risk by nocturnal avian predators reduced the activity of carabid seed predators by 50%, but the net effect of small mammals on seed removal was neutral.

Finally, in chapter 5, I directly evaluated the utility of seed predation by measuring the effects of seed predators on weed emergence. I simulated seed rain of common lambsquarters, and found that seed predators overcame intense propagule pressure and reduced the germinable seedbank. I found 38% fewer seedlings in seed-augmented plots where seed predators had access, compared to plots where they were excluded. Minimal differences between differential exclusion of vertebrate and invertebrate seed predators

suggest that the effect of vertebrates on seed predation is neutral, corroborating evidence from chapter 4.

Together, these five chapters enumerate multiple interacting drivers of tropic cascades, with insights of both basic and applied importance. I learned that predator avoidance and intraguild predation interact and simultaneously shape trophic ecology, with distant downstream implications. Because each process is common in nature, it is important to integrate both in future predictions of trophic dynamics. Provisions of vegetative cover can promote weed biological control by both attracting more seed predators and facilitating per-capita seed consumption. While vegetation may also facilitate intraguild predator events, these effects are minimal compared to the strong positive effects of cover on seed predation overall. Thus, cover crops and forage crop rotations can be powerful tools to promote weed biological control, among the numerous other benefits they provide.

CHAPTER 1.   A META-ANALYSIS OF SEASONAL DYNAMICS AND
SPECIES-SPECIFIC IMPACTS OF WEED SEED BIOLOGICAL CONTROL
BY VERTEBRATE AND INVERTEBRATE SEED PREDATORS

1.1   ABSTRACT

The body of research documenting ecosystem services performed by weed seed

predators in crop environments has grown quickly in the last 10 years, due to renewed

interest in ecological weed management tools. Important seed predator taxa include

vertebrates (small mammals), and invertebrates (ground beetles and crickets), and while

many studies evaluate the relative importance of vertebrates and invertebrates, the

magnitude of their impact seems to be site and species specific. These context-dependent

effects suggest the need for a quantitative review of local factors that drive seed

predation, as well as the seasonal dynamics of weed seed predation. We used meta-

analysis to evaluate the effects of vertebrate exclusion on seed removal across season and

weed species, and present a quantitative, descriptive summary of seed removal rates

across crop types varying in habitat quality. Synthesizing 25 studies, we learned that

vertebrates have a relatively moderate impact on seed removal overall during the active

growing season, while invertebrates are responsible for >50% of seed removal in most

studies. Vertebrate seed predation is highest during the summer, whereas invertebrates

dominate seed predation in the spring and autumn. Seed predation by vertebrates and

invertebrates is very low in fully exposed environments, but varies substantially between

crop habitats. These trends help identify and promote crop environments where seed predators can have an agriculturally relevant impact on weed pressure.

Keywords: Weed seed predation, Carabidae, *Peromyscus*, cover crops, biological control

1.2 INTRODUCTION

Ecosystem services performed by animals who consume weed seeds have warranted steady research attention for more than thirty years (Lund and Turpin 1977). Recently, interest in weed seed predation has mounted due to a renewed focus on ecological weed management strategies, particularly in organic systems where weed suppression is especially challenging (Landis et al. 2005). Both vertebrates (e.g. birds and rodents) and invertebrates (e.g. ground beetles and field crickets) are important weed seed predators in annual crop environments. While many studies use seed removal assays to evaluate the importance of each group, the magnitude of their seed predation seems to be region and site-specific (Davis et al. 2013, Westerman et al. 2003, Brust and House 1988). These context-dependent effects suggest the need for a quantitative review of local factors that drive seed predation. For example, cropping systems that provide groundcover may enhance weed seed predation by providing diverse food resources, shelter, and refuge. Yet, these findings are not consistently demonstrated experimentally (e.g. Ward et al. 2011). Also, timescales and focal species very considerably across studies. Seasonal climate and specific qualities of weed seeds may explain variation observed in seed removal. While specific mechanisms of seed predator recruitment are not yet clear in the body of literature, we can extract important trends that will help to identify and promote environments where seed predators are likely to have an agriculturally important impact on weed pressure.

1.2.1   Seed predator taxa

The most commonly examined vertebrate weed seed predators in crop systems are nocturnal mice. *Peromyscus* spp. are most commonly captured in North America (Brust and House 1988), and *Apodemus* spp. are most common in Palearctic regions (Westerman et al. 2003). *Mus musculus* is also a consumer of weed seeds, but is less common in arable systems (Whitaker 1966). Seeds constitute an important part of avian diets, however, there is little evidence suggesting their importance in regulating the weed seedbank in crop fields (Holmes and Froud-Williams 2005, Mauchline et al. 2005, but see Navntoft et al. 2009). Birds apparently do not forage effectively in a crop canopy (Mauchline et al. 2005), but may be important seed predators during the winter and early spring (Holmes and Froud-Williams et al. 2005, Holland et al. 2008), when fields are bare.

Invertebrates, usually carabid beetles, are cited as the dominant weed seed predators in many temperate agricultural systems (Gallandt et al. 2005, Menalled et al. 2007, Ward et al. 2011, Fischer et al. 2011, Fox et al. 2013). Crickets, mostly from the genus *Gryllus,* are also important seed predators (O'Rourke et al. 2006, Westerman et al. 2008, Carmona et al. 1999), although their prevalence may be under-represented in the current body of literature. Due to their leaping habit, crickets have a lower probability of capture using the typical pitfall sampling strategy (Barney et al. 1979). Like crickets, ants escape detection with pitfall traps as well. *Lasius* spp. and *Pheidole* spp. have been observed removing weed seeds (Brust and House 1988) in temperate systems, however except for some harvester ant species which completely consume weed seeds (Baraibar et

al. 2009), it is unclear as to whether or not ants act as seed predators, dispersers, or have

neutral effects on weed population dynamics (VanderWall et al. 2005, Lundgren 2009).

Pitfall sampling is used ubiquitously to quantify invertebrate weed seed predator

communities, however it is inadequate for measuring abundances of many seed-feeding

invertebrate species. Also, failure to account for imperfect detection is a pervasive

problem across ecological disciplines (Kellner and Swihart 2014), and it substantially

weakens efforts to connect natural enemy populations with biological control.

Additionally, fluctuating temperatures bias pitfall trap captures (Saska et al. 2013).

Structurally complex habitats impair insect movement, bringing attention to a long-

acknowledged problem with bias related to vegetative structure in pitfall data

(Greenslade 1964). For these reasons, this meta-analysis focuses exclusively on weed

seed biological control, rather than seed predator communities.

1.2.2 Environmental resources:

Vegetative habitat resources may enhance seed predation (Gallandt et al. 2005,

Meiss et al. 2010), but they may also facilitate competition or intra-guild predation

among vertebrate and invertebrate seed predators (Birthisel et al. 2014). Because most

seed predators are omnivorous (Lundgren 2009), it is important to consider how feeding

habits of different taxa vary temporally with resource availability. Vertebrate and

invertebrate seed predators have different foraging strategies, and likely have taxa-

specific effects on the weed seedbank that are mediated by food preferences (Westerman

et al. 2008).

The wealth of research documenting weed seed predation in crop environments

has yielded several qualitative reviews that suggest tools to enhance seed predator

recruitment (Lundgren 2009, Landis et al. 2005, Menalled et al. 2002, Tooley and Brust 2002). However, qualitative reviews in this field are somewhat limited in utility by the site-specific nature of seed predation dynamics. A quantitative review can identify trends across studies that make weed biological control more predictable across weed species, environmental, and temporal gradients. We use a meta-analytic approach to evaluate the relative importance of vertebrate and invertebrate seed predators on seed removal across seasons, weed seed size classes, and commonly surveyed weed species. We predicted that vertebrate seed predators would have stronger effects on large-seeded weed species (Munoz and Bonal et al. 2008), and that their impact would be greatest during the autumn, when summer-annual weeds senesce. Unlike invertebrates, vertebrates have been shown to have density-dependent responses to seed resources (Westerman et al. 2008, Janzen 1970). We also present descriptive summaries of seed removal rates for both invertebrate and vertebrate taxa across seasons and in crop environments that vary in habitat structure. This comprehensive, quantitative, review will assess multiple variables impacting seed predators' capacity to limit flux to the weed seedbank.

1.3   METHODS

1.3.1   Literature search

We began with a list of studies included in existing qualitative reviews of weed seed biological control (Tooley and Brust 2005, Lundgren 2009). We searched forward and backward citations within each article from the initial list on Google Scholar and Web of Science, and also performed keyword searches using "weed seed predat*". Those which measured weed seed predation services in crop systems by comparing open seed removal assays (measuring total seed losss) with treatments that excluded vertebrate seed

predators (measuring invertebrate seed removal only) were included. Five authors were contacted who supplemented data that enabled the inclusion of their studies in the meta-analysis, and one unpublished study (Chapter 4) was added. Twenty-one papers that reported sample sizes and variance were used in the meta-analysis. Four additional papers that reported means without variance were included in a descriptive summary of seed predation rates across seasons and in varying crop types.

1.3.2   Data collection

If mean proportions of seeds removed by vertebrates and invertebrates were not available in tables within a manuscript, we gathered individual data points by digitizing plots and measuring means and variance for multiple timepoints using an online plot digitizer (Huwaldt 2005; http://plotdigitizer.sourceforge.net). Most studies reported seed removal rates for open treatments and vertebrate exclusion treatments. Some reported the proportions of seed removal directly attributable to vertebrates and invertebrates. Both styles of presentation were converted to a standard proportion of seeds removed in open and vertebrate exclusion treatments. To summarize seed removal rates across crop types and seasons, all measurements of seed removal were converted to a nightly proportion of seeds removed by vertebrates and invertebrates. We also collected several kinds of meta-data including weed species identity, crop environment, Julian day, season (spring, summer, and autumn, according to vernal and autumnal equinoxes), year, study and site identity. Average seed weights were collected from literature (Table 1.2), and these were used to divide weed species into seed size classes. Large seeds were >2mg; medium seeds were between 1 and 2 mg, and small seeds were <1mg.

We calculated an effect size for each sample date, crop environment, and seed species examined within studies. To avoid pseudoreplication in our meta-analyses, effect sizes were pooled by year. We considered each year and site of a study to be independent measures, giving greater weight to multi-year, site-replicated projects. For analyses relating to temporal variation in seed removal, repeated measures were pooled by season.

1.3.3   Statistical analysis

The effect size metric used in this analysis was Hedges g, derived from Cohen's d with a correction factor J, for small sample sizes (Borenstein et al. 2009). We calculated Hedges' g as the mean difference between the proportions of seeds removed behind a vertebrate exclosure cage and from an open control, divided by the pooled variance of both values. When possible, seed removal proportions were corrected for abiotic causes of seed loss, otherwise they were assumed to be uniform across treatment groups. A negative effect size in this system means that vertebrate exclusion reduces overall seed removal, thus stronger negative effect sizes imply a greater relative importance of vertebrate seed predators. Weak effects were close to zero, moderate effects were around -0.5, and strong effect sizes were lower than -1 (Borenstein et al. 2009).

We used our meta-dataset to ask three questions about the effects of vertebrate exclusion on seed predation: 1) How does the impact of vertebrate seed predators vary across the growing season? 2) Which weed species do vertebrate seed predators impact? 3) How does seed size predict the impact of vertebrate seed predators? Because all effect sizes were negative (vertebrate exclusion almost always reduces overall seed removal), we ran our analyses in R on the absolute value of Hedges g (invertebrates only – total seed removal). Because the distribution of effect sizes did not meet normality

assumptions, we used quasi-poisson generalized linear models. We ran separate glms for each question listed above. Hedges g was the response variable for each model, and season, weed species, or seed size were categorical predictor variables, blocked by study identity. We used t tests to determine if mean effect sizes for each of the categorical variables significantly differed from zero. We visually examined the potential of publication bias in this meta-analysis using a funnel plot, but found little evidence of bias affecting our results.

1.4   RESULTS AND DISCUSSION

1.4.1   Seasonal variation*:*

Invertebrates were responsible for >50 % of total weed seed removal in the spring and autumn (Fig. 1.1). These periods correspond to activity peaks for invertebrate seed predators, which are either active in the spring or fall, depending on the species (Kotze et al. 2011). Typically there is a mid-summer reduction in invertebrate seed predator activity, concurrent with high temperatures and low humidity (Kirk 1973). However these activity patterns, largely documented with pitfall trapping, are incongruent with invertebrate seed removal, which was not apparently lower in the summer. In fact, overall seed removal trended highest during the summer (Fig 1.2).

Vertebrates contributed more to overall seed removal in the summer, compared with later in the fall (Fig 1.1; t=2.686, df=62, p=0.009). During the spring, the mean effect size was not significantly less than zero (Fig 1.2; t =-2.1714, d=8, p=0.9692), suggesting weak and highly variable contribution of small mammals to seed predation services during this period. Relatively few studies examine seed predation in the spring (Fig 1.1), and future work targeting seed predator foraging activity early in the growing

season may better clarify patterns. The absence of vertebrate effects on seed removal during the spring may also reflect the emergence of alternative, or more preferable food resources (Fig 1.2). Indeed, the temporal patterns observed here are consistent with foraging strategies of rodents active in agricultural systems, which prey heavily on lepidopterous larvae and other arthropods during the months they are available and shift to seed-feeding in the winter (Mumford and Whitaker 1982, Flick 2013). Very little work has been done to examine seed predation during the winter, despite the fact that seed predation by rodents is quite substantial during this period (Davis et al. 2013, Williams et al. 2009, Marino et al. 1997). Williams et al. (2009) found greater than 75% removal for velvetleaf and foxtail seeds between November and March over three consecutive winters. The dearth of studies on overwinter seed predation limits our ability to predict ecosystem services during this period.

Early autumn marks the peak activity period for most invertebrate seed predators, which provide the majority of weed seed predation services during the critical period of summer-annual weed senescence. Small mammal densities are also highest in autumn (Green 1978, Mumford and Whitaker 1982), at the end of the major breeding season (April-November), before late- winter dieback (Vessey and Vessey 2007). Despite the concurrent activity peaks, seed removal rates did not appear to increase during the autumn (Fig 1.1). The convergence of peak activity periods and the common use of vegetative cover by vertebrate and invertebrate seed predators suggests the possibility of invertebrate predation by small mammals, which may explain the less-than additive effects of both seed predator groups.

1.4.2   Crop environment:

Bare crop environments (recently tilled or recently harvested areas) had the lowest nightly seed predation rates across studies (Fig 1.3). Some invertebrates have temperature and humidity requirements that confine their foraging activity to vegetated areas (Saska et al. 2010), and many respond to increased food availability associated with vegetative habitats (Diehl et al. 2012). Small mammals' use of cover is largely determined by perceived predation risk (reviewed in Brown and Kotler 2000), and this varies according to indirect cues of predation risk like moonlight (Abramsky et al. 2002, Orrock and Danielson 2004, Davis and Raghu 2010) as well as seasonal changes in predator abundance (Brown 1989). Vertebrate contributions to seed removal were greater or equivalent to those of insects in forage and broadleaf crops (Fig 1.3), which are both characterized by a fully-closed canopy in the summer and early autumn, providing refuge from nocturnal avian predators of small mammals. Nightly seed removal was rather low in grassy, perennial margins (Fig 1.3), which was surprising, as perennial grass banks are often recommended for implementation as refuge habitat for overwintering seed predators (Griffiths et al. 2008). Low seed removal rates in grass suggest that effects of vegetative cover on ecosystem services are complex, and may depend on the structural quality (i.e. permeability) of the refuge habitat (Frampton et al. 1995).

Total seed removal was similarly high in corn, small grains, forage, and broadleaf crops (Fig 1.3). These cropping systems all represent a wide variety of structural quality, tillage, and disturbance frequency, making it difficult to draw conclusions about specific habitat attributes that promote seed predation. Seed-feeding carabids seem to be more active under a vegetative canopy (Gallandt et al. 2005, Meiss et al. 2010, Fox et al. 2013,

Altieri et al. 1985, Diehl et al. 2012), but cover associations vary for different species of invertebrate seed predators. For example, *Pterostichus melanarius Illiger* is one of the most common, highly mobile carabid species in Europe and North America. While often predatory, *P.melanarius* also readily feeds on seeds (Tooley et al. 1999), and forages more in field crop environments than in areas with dense groundcover (Powell et al. 1985).

1.4.3 Seed preference

Vertebrate exclusion had the greatest effect on giant ragweed (Fig 1.4), the species with the most massive seeds of any examined in this meta-analysis. Unless seeds are imbibed, invertebrates are somewhat constrained to forage on seeds with dimensions smaller than their mandibular widths (Lundgren and Rosentrater 2007, White et al. 2007). Thus vertebrates are likely responsible for most biological control of larger dormant seeds. Further, small mammals frequently prefer large-seeded species with higher carbohydrate content and lower foraging investment per seed (Munoz and Bonal 2008). While rather large-seeded, velvetleaf (*Abutilon theophrasti* Medik*)* was not disproportionately affected by vertebrate seed predators, possibly due to its extremely hard seedcoat (Davis et al. 2008), and longer handling time. The weak effect of vertebrate seed predators on *A. theophrasti* substantially diminished the overall effect of vertebrates on large-seeded weed species (Fig 1.5).

Vertebrate exclusion also strongly reduced foxtail seeds (*Setaria spp.*; Fig 1.4), which are medium-sized, and highly preferable to crickets in lab-feeding trials (Lundgren and Rosentrater 2007). Grass seeds are clearly important components of the diets of small mammals in arable land (Whitaker 1966, Houtcooper 1978,a,b), as they are often the

most abundant seed resource. If grass seeds are commonly fed on by both vertebrates and invertebrates, and these foraging behaviors overlap temporally, it is possible that the two groups may be competitors for seeds in agroecosystems. Although competition between mammals and invertebrates for seed resources has been demonstrated in desert ecosystems (Brown and Davidson 1977), the possibility has never been explored in annual crops. The strong effect of vertebrates on removal of medium-sized seeds compared to both large and small (Fig 1.4; $t=2.598$, $df=61$, $p=0.01$) was largely driven by the pronounced impacts on *Setaria spp.*

Invertebrates were responsible for most seed predation of smaller-seeded weed taxa like pigweed (*Amaranthus retroflexus* L.), lambsquarters (*Chenopodium album* L.) and chickweed (*Stellaria media* L.), and small mammals contributed less to biocontrol for these species (Figs 1.4 and 1.5). Lambsquarters and pigweed have been shown to be highly palatable species to both crickets and carabids in choice feeding trials (Lundgren and Rosentrater 2007). Still, the mean effect size was significantly less than zero for all species (Fig 1.5, $t=-6.245$, $df=64$, $p=<0.001$), or seed size (Fig 1.4; $t=-6.876$, $df=66$, $p=<0.001$), suggesting that small mammals still contribute to seed loss for small seeds, despite contrasting preferences.

1.4.4   Conclusions and synthesis

Reduced tillage programs are commonly recommended to capitalize on weed seed predation because they allow for senesced seeds to remain on the soil surface, accessible to epigeal seed predators for a longer period of time (Westerman et al. 2006). No-till crop environments may further enhance seed predation if benign levels of weed growth are tolerated because even sparse vegetation provides suitable refuge for both vertebrate and

invertebrate seed predators (Tew et al. 2000, Hough-Goldstein et al. 2004, Ward et al. 2011). Further, combining a no-till strategy with cover-crops may create an abundance of refuge habitat and a more stable foraging environment, more closely approximating the optimal temperature and humidity levels for seed consumption (Saska et al. 2010). Nevertheless, it is clear from this review that not all vegetative resources are of equal value as refuge to seed predators (Fig 1.3).

While many seed predators use vegetative cover as refuge, we found that effects of cover on seed predation depend on the qualities of specific cover types (Fig 1.3). Refuge habitat should facilitate seed-feeding because it harbors higher densities of active seed predators (Gallandt et al. 2005), and phenomena that potentially dismantle relationships between predator density and seed removal warrant investigation. Seed removal rates are products of not only seed predator density and surface habitat structure, but background seed density (i.e. predator hunger and satiation; Saska et al. 2008), temperature (Saska et al. 2010), and other potential confounding factors, like predation risk. Despite the persistence of context-dependent effects on weed seed predation, achieving a more comprehensive understanding of the multiple drivers of seed removal will promote awareness of ecosystem services provided by seed predators, and encourage management strategies that promote their activity and survival.

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Table 1.1 List of studies included in this meta-analysis that examined vertebrate and invertebrate weed seed predation. Dominant predator taxa identified were responsible for >50% of seed predation in the study.

	Study	Crop	Predator taxa	Location
1	Baraibar et al 2009	barley	ants	Spain
2	Birthisel et al 2013	various	carabids	Maine, USA
3	Blubaugh et al 2015	forage	carabids	Indiana, USA
4	Brust and House 1988	soybean	carabids	North Carolina, USA
5	Cardina et al 1996	corn	rodents	Ohio, USA
6	Cromar et al 1999	corn/soybean	carabids	Ontario, CAN
7	Davis and Raghu 2010	corn	unk	Illinois, USA
8	Deadlow et al 2012	winter cereal	carabids	Germany
9	Fischer et al 2011	winter cereal	carabids	Germany
10	Fox et al 2013	soy/corn/forage	carabids	North Carolina, USA
11	Gaines and Gratton 2010	vegetable/margin	rodents	Wisconsin, USA
12	Gallandt et al 2005	vegetable rotation	carabids	Maine, USA
13	Harrison et al 2003	no till corn	unk	Ohio, USA
14	Marino et al 1997	corn	unk	Michigan, USA
15	Mauchline et al 2005	spring barley	carabids	UK
16	Meiss et al 2010	forage	carabids	France
17	Menalled et al 2000	corn	unk	Michigan, USA
18	O'Rourke et al 2006	soy/triticale/alfalfa	crickets	Iowa, USA
19	Povey et al 1993	cereal margin	unk	UK
20	Spafford Jacob et al 2006	winter cereal	ants	W. Australia
21	Ward et al 2011	sweet corn	carabids	Pennsylvania, USA
22	Westerman et al 2003	winter cereal	rodents	Holland
23	Westerman et al 2008	corn	crickets	Iowa, USA
24	Westerman et al 2010	sugar beet	rodents	Holland
25	White et al 2007	no till corn	carabids	Michigan, USA

Table 1.2 List of seed weights collected from published literature for weed species included in the meta-analysis.

Species	Common name	Seed mass (mg)	Reference
Chenopodium album	Lambsquarters	0.7	Gallandt et al 2005
Amaranthus retroflexus	Pigweed	0.53	Gallandt et al 2005
Setaria faberi	Foxtail	1.65	Gallandt et al 2005
Abutilon theophrasti	Velvetleaf	7.39	Gallandt et al 2005
Brassica kaber	Wild mustard	1.76	Gallandt et al 2005
Galinsoga quadriradiata	Hairy galinsoga	0.14	Gallandt et al 2005
Ambrosia trifida	Giant ragweed	46.09	Lundgren and Rosentrater 2007
Ambrosia artemisifolia	Common ragweed	5.34	Gross 1990
Avena fatua	Wild oat	2.1	Westerman et al 2003
Stellaria media	Chickweed	0.4	Westerman et al 2003
Gallium aparine	Cleavers	6.64	Westerman et al 2003
Taraxacum officinale	Dandelion	0.48	Honek et al 2007
Viola arvensis	Violet	0.46	Honek et al 2007
Capsella bursa-pratoris	Shepherd's purse	0.23	Honek et al 2007
Bromus sterilis	Brome	8.37	Thompson et al 1993
Senna obtusifolia	Sicklepod	20.2	Brust and House 1988
Datura stamonium	Jimsonweed	8.2	Brust and House 1988
Apera spica-venti	Silky bent grass	0.017	Warwick, Thompson & Black 1987
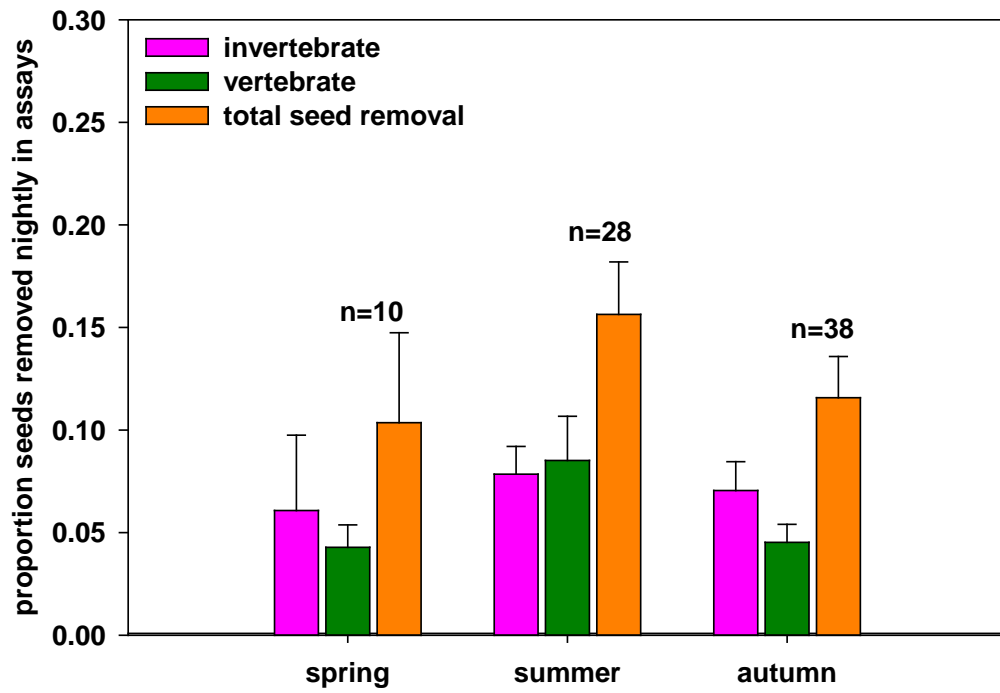
Figure 1.1 Pooled means (+SE) of nightly vertebrate and invertebrate seed predation rates across seasons from 21 studies.
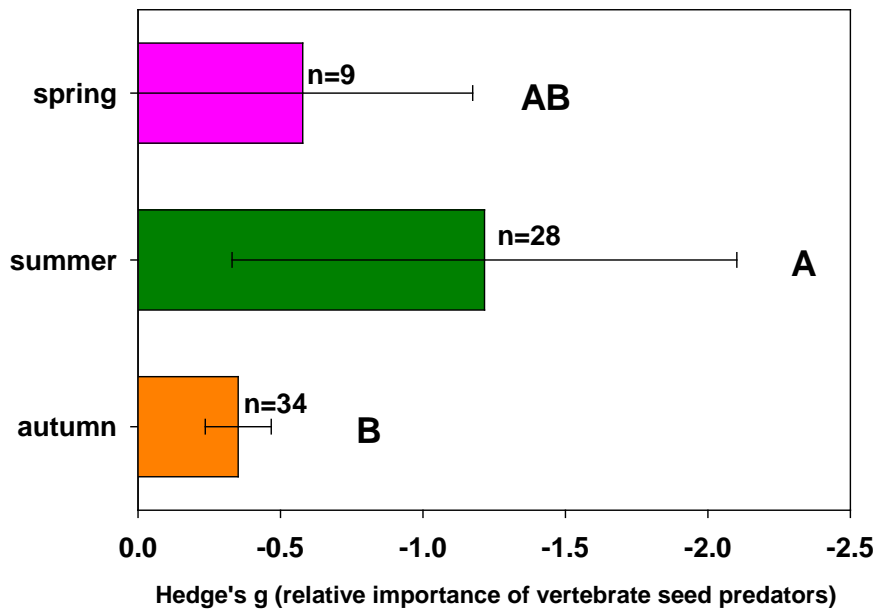
Figure 1.2 Mean effect size (+ 95% confidence interval) for Hedges'g, which indicates the importance of vertebrate seed predators by season across 21 studies. Different letters indicate significant differences between groups. Effect sizes are negative because they represent the magnitude of the reduction in seed removal rates when vertebrate seed predators are excluded.
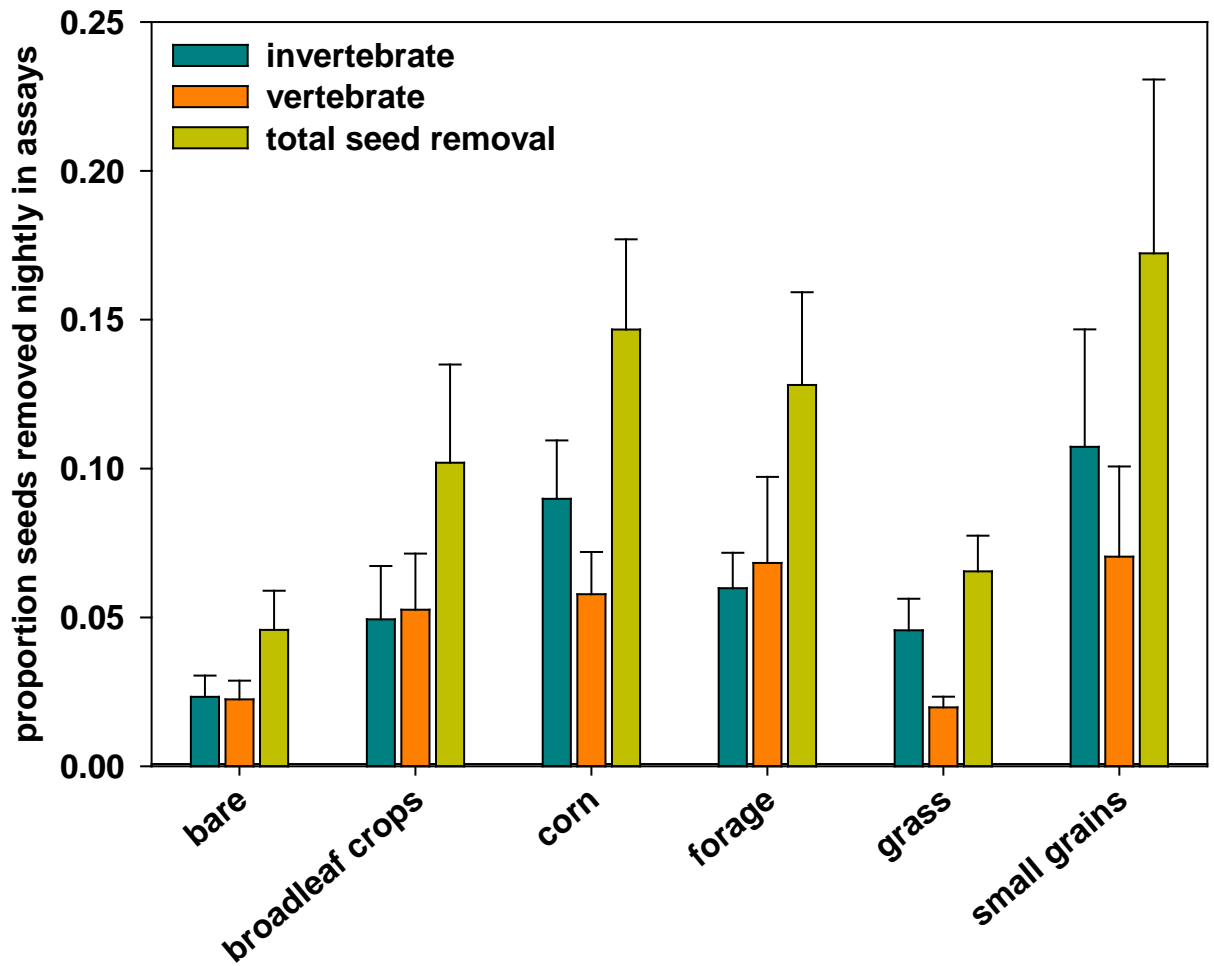
Figure 1.3 Pooled means (+ SE) of nightly vertebrate and invertebrate seed predation rates across 21 studies in crop systems with varying habitat structures. Forage included red clover, alfalfa, and timothy.
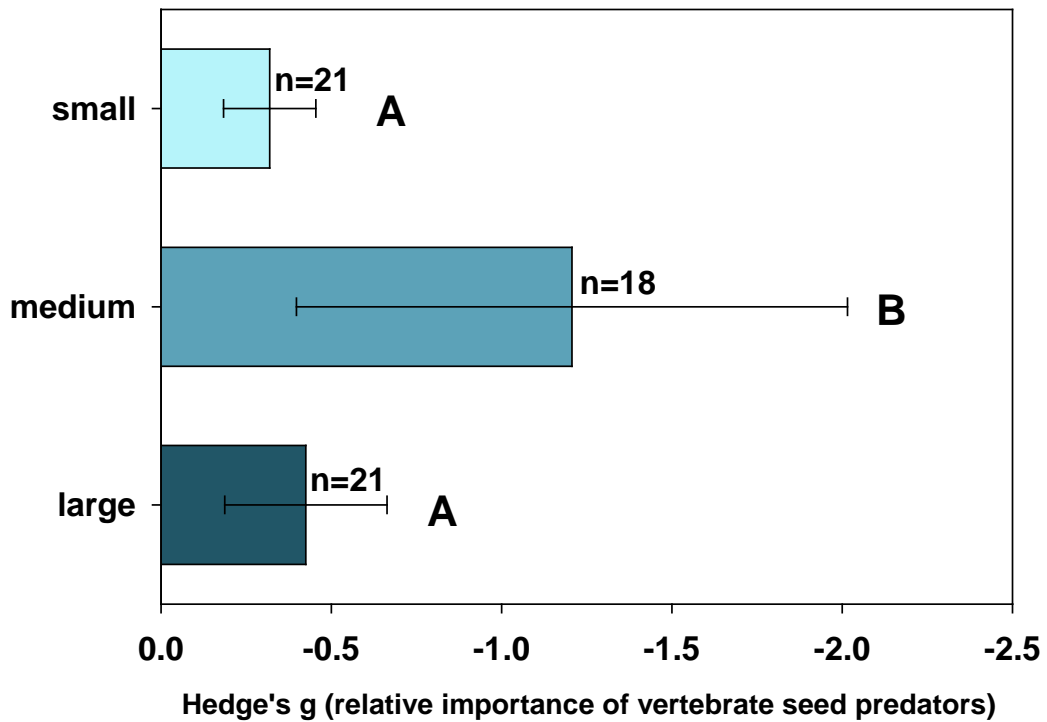
Figure 1.4 Mean effect size (+ 95% confidence interval) for Hedge's g across 20 studies, grouped by weed seed weight class. Large seeds were >2mg; medium seeds were between 1 and 2 mg, and small seeds were <1mg. Effect sizes are negative because they represent the magnitude of the reduction in seed removal rates when vertebrate seed predators are excluded.
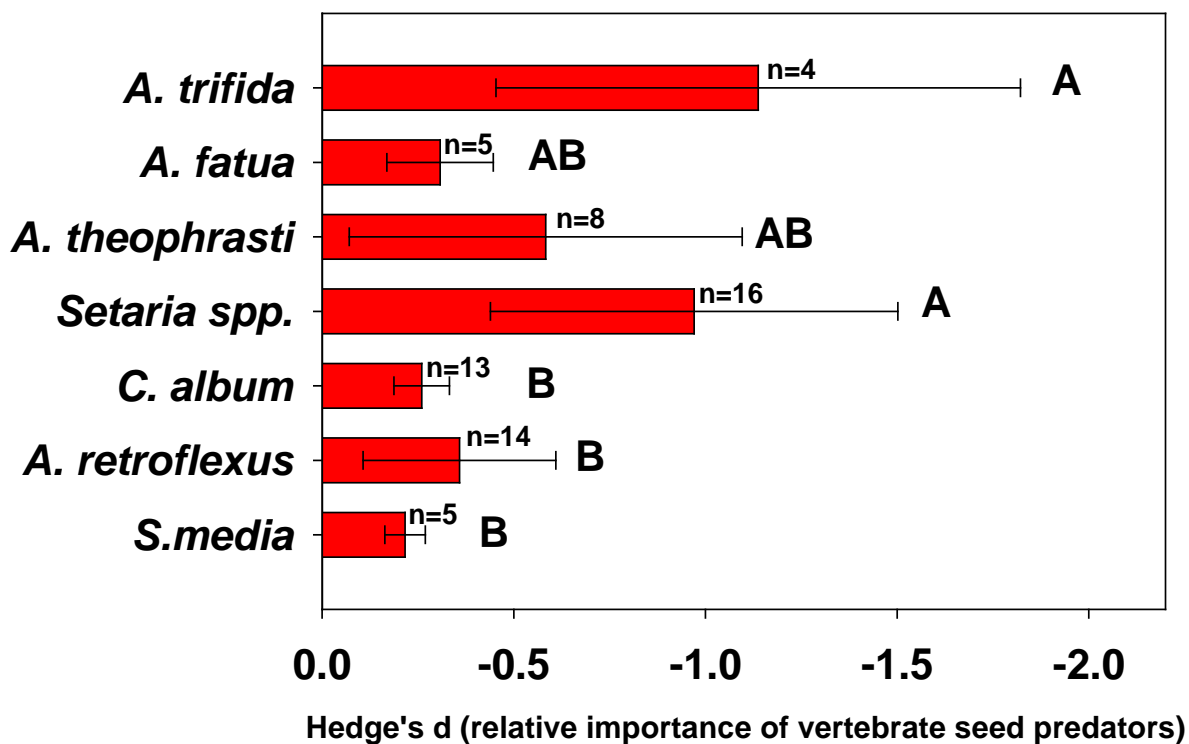
Figure 1.5 Mean effect size (+ 95% confidence interval) for each of the most commonly examined weed species across 14 studies that reported weed species identities. Species are presented in order of seed weight, heaviest to lightest. Effect sizes are negative because they represent the magnitude of the reduction in seed removal rates when vertebrate seed predators are excluded.

CHAPTER 2.   TILLAGE COMPROMISES WEED SEED PREDATOR ACTIVITY ACROSS DEVELOPMENTAL STAGES

2.1   ABSTRACT

Granivorous ground beetles (Coleoptera: Carabidae) are ubiquitous throughout temperate agricultural systems, and reduce weed seedbanks. However, trade-offs may exist between tillage frequency and ecosystem services of invertebrate seed predators, especially those in the larval stages, which have relatively poor resistance to disturbance. While much research has focused on adult activity patterns and the conservation biocontrol services they provide, almost nothing is known about carabid biology and habitat requirements during larval stages, despite the fact that adult recruitment is determined by factors that promote larval survival. We present data on larval and adult *Harpalus pensylvanicus* DeGeer, a common weed seed predator across North America, from two experiments examining its activity density across tillage and cover-cropping treatments in organic tomato systems. Larvae emerged 4–6 weeks after the adult activity peak, and larval activity density was up to 10 times higher in no-till crop environments than in cultivated areas. After a long disturbance interval, seasonal cultivation had no effect on foraging activity of adults, but reduced larval activity density in both experiments. Additionally, larvae positively correlated with living weed biomass in no-till treatments, suggesting the importance of plant-based resources in oviposition site choice. Compared with adults, larvae are relatively immobile and vulnerable to

disturbance; thus, weed management strategies that rely on frequent cultivation may undermine the ecosystem services provided by granivorous insects.

2.2   INTRODUCTION

Increasing demand for produce raised without chemical inputs drives agroecologists to develop stronger, ecologically based, cultural tools for farmers to manage weeds. An ecological approach to weed management combines several tactics including crop rotation, tillage, cover cropping, and conservation biological control as an alternative to herbicides (Westerman et al. 2005). Of these tactics, tillage is most frequently used in herbicide-free systems (Liebman and Davis 1999). However, tillage also reduces habitat stability, which may ultimately disrupt colonization and/or survival of beneficial insects that act as weed seed predators. This creates a potential trade-off between mechanical weed suppression and biocontrol. For instance, invertebrate seed predators can reduce seedbanks and affect weed population dynamics (Davis et al. 2003, Westerman et al. 2006, Bohan et al 2011), but suffer high mortality due to heavy tillage, which destroys food and habitat resources (Purvis and Fadl 2002, Holland and Reynolds 2003). A comprehensive knowledge of the ecological costs of cultivation demands a detailed understanding of this trade-off over biocontrol agents' entire life histories.

Ground beetles (Coleoptera:Carabidae) are dominant seed predators in many field crop systems, and numerous studies have tested the effects of cover and tillage on seed predation by adults (Gallandt et al. 2005, Pullaro et al. 2006, Shearin et al. 2008, Meiss et al. 2010, Ward et al. 2011). Despite this wealth of data on carabid adults, almost nothing is known about distributions of larvae in heterogeneous environments (Luff and Larsson 1992), largely because they are cryptic and challenging to identify (Lovei and Sunderland

1996). The sparse information on larval phenology and life history of seed-feeding

carabids in crops is mostly observational (Kirk 1972a,b, 1973) or lab-based (Jorgenson et

al. 1997, Hartke et al. 1998, Saska 2005), and few experimental studies have documented

larval activity in field investigations (Luff 1980, Traugott 2001, Noordhuis et al. 2001

Purvis and Fadl 2002, Holland et al. 2004, 2007, Frank et al. 2010, Lundgren et al 2013).

     Harpalus pensylvanicus DeGeer is the most common carabid seed predator in

many agricultural systems across North America (Barney and Pass 1986 Davis and

Liebman 2003, Lundgren et al. 2006, Ward et al. 2011, Fox et al. 2013), with a peak

activity period in the autumn (August-September) that directly coincides with the

senescence of many summer annual weeds (Kirk 1973). 1st instars (Fig 2.1) emerge in

late autumn, ca. 6 weeks after the adult activity peak, and are identifiable by their

enlarged heads and mandibles (Tomlin 1975), acuminate laciniae, unequal claws

(Bosquet 2010), and a signature shape of the frontal margin (Kirk 1972a). All instars of

H. pensylvanicus are surface-active, and can be captured in pitfall traps from late October

until well past the first frost (Fig 2.2). They actively forage on eggs and small,

subterranean larvae (Kirk 1973) for about 4 weeks before overwintering in small burrows

where they cache weed seeds (Kirk 1972b). Larvae are relatively immobile and

vulnerable compared to adults, and most mortality occurs before pupation (Kromp 1999).

Due to high larval mortality, adult recruitment and weed seed biocontrol may be largely

driven by cultural management strategies that promote larval success (Holland et al

2007).

     Here, we report on larval activity patterns of *H. pensylvanicus* within two separate

field experiments that are part of a larger, interdisciplinary organic agriculture research

project comparing various cultural weed management strategies of particular interest to vegetable growers (Butler 2012). In the first, we compare four weed management regimes in a market tomato system that include tillage, plastic mulch, living mulch and roller crimped rye mulch. In the second, we again compare tillage practices, as well as a variety of fall cover crop species and the effects of living weed biomass. We hypothesized that larvae would be disproportionately active in no-till treatments, particularly those containing a killed fall cover crop, which provides insulating thatch and additional larval food resources. We also document emergence times for *H. pensylvanicus* larvae in the Midwestern United States, and discuss cultural treatments that may enhance overwintering survival.

2.3   METHODS

We conducted both experiments at the Purdue University Meigs Horticulture Research Farm near Lafayette, IN (40° 17' 15" N, 86° 53' 1" W) using organic crop management practices.

2.2.1   Experiment 1

Experimental Design

This experiment was nested within a larger project incorporating interdisciplinary perspectives in a side-by-side trial of several cultural weed management strategies. We compared carabid activity across cover crop and tillage treatments in tomato (*Solanum lycopersicum* cv. 'Fraisers Gem') over the 2011 growing season. We used a randomized complete block design, creating four 6mx6m treatment plots per block, with four crop rows spaced 1.8 m apart within each plot, arranged in five randomized blocks with 4.5m margins between plots and blocks that were tilled bi-monthly. The experimental matrix

was in organic transition; it was planted with red clover (*Trifolium pratense* L.) over the two previous years, bordered on three sides by an untilled perennial grass margin. The first treatment (TILL) represented standard practices for organic fresh-market tomato production. Plots were disc-plowed in May 2011 at a depth of 15 cm, and plastic mulch was used to suppress weeds within rows. Cultivation controlled weeds between rows, and occurred on June 8, July 6, July 27 and August 10. In the second treatment (TILL+CLOVER), tomatoes were transplanted into plastic mulch 0.9m apart, cultivated as needed for 8 weeks between rows, then planted with a crimson clover (*Trifolium incarnatum* L.) cover crop at 35 kg seeds per ha. In the third treatment (NO-TILL RYE), a cereal rye (*Secale cereale* L.) cover crop was drill-seeded at a rate of 135 kg seeds per ha in fall 2010 and was roller-crimped in on June 8, 2011. Tomatoes were no-till transplanted into the rye mulch 48 hrs after crimping. The last treatment (STRIP-TILL RYE +CLOVER) was similar to NO-TILL RYE, but the area between rows was tilled and planted with red clover on June 7, 2011 (rye mulch was left within rows). Instead of cultivation, weeds were managed in the NO-TILL RYE and STRIP-TILL RYE + CLOVER treatments by mowing between rows on July 6, July 27, August 8, and September 8. As a forage crop, red clover tolerates mowing well, with vigorous regrowth. Weed management activities were performed with a BCS 722 walking tractor in all treatments using mower and tiller attachments.

Sampling

From July-November 2011, we collected carabids weekly from two pitfall traps (plastic deli cups 15 cm in diameter) filled with 1cm soapy water linked by a 75x8cm aluminum flashing barrier in the center row of each plot. Barriers were placed at 45 degree angles

between crop rows. As night time temperatures fell in mid-September, we collected traps

every two weeks, and placed carabid adults and larvae in vials with 70% ethanol. Pitfall

traps were open continuously during the sampling periods. Heavy rain events caused

traps in tilled plots to flood, so exact collection periods varied with inclement weather.

We identified adults and larvae using Bosquet 2010 and Kirk 1972a, and adults were

confirmed with specimens in Purdue's Entomological Research Collection (PERC).

Voucher specimens were subsequently deposited in PERC. We identified adults to

species and larvae to the tribe level. Although most 3rd instars were identified as *H.*

pensylvanicus, 1st and 2nd instars are difficult to identify beyond tribe. Thus, we pooled

all specimens in one taxonomic group within the tribe Harpalini, which was likely

dominated by *H. pensylvanicus*. All genera within the Harpalini are opportunistically

granivorous as adults (Lundgren 2009), and can contribute to weed seed predation

services in crop environments.

2.2.2   Experiment 2

Experimental Design

In 2012, we compared four common fall cover crops (rye, rye/vetch, oriental mustard,

and a fallow control) and two tillage treatments (no-till and spring tillage) in a

randomized split-plot design. In September 2011, the plots were disc-tilled and the four

cover crop types were planted in four replicate blocks consisting of 15x9m main plots

with 4.5m margins, which were tilled bi-monthly. Mean dry stand weights for the rye,

rye/vetch, and mustard cover crop treatments were 303, 342, and 444 g/m^2, respectively

at termination. The experimental matrix was fallow for 10 years previously, and

surrounding margins as well as adjacent crop areas were tilled in spring 2012 and planted

with sorghum-sudangrass (*Sorghum × drummondii* Steud). In May 2012, plots were split in half; one subplot was flail-mowed and tilled before tomatoes were transplanted (cv. 'Brandywine') 0.9m apart. In the other subplots, cover crops were flail mowed and left as mulch on the soil surface between rows. There was no buffer between the subplot tillage treatments. Black plastic controlled weeds within rows and mowing occurred between rows on June 27th. Very little weed control was required due to extreme drought conditions in 2012. In mid-September, cultivated subplots were tilled again, well before the larval emergence period.

Sampling

Collection and identification methods were similar to Experiment 1 except that pitfall traps were dry and not barrier linked. Experiment 1 initially targeted adults, whose capture rates are greatly improved by barrier-linked trapping (Winder et al 2001), and larvae were collected incidentally. Larvae were the intended subject of sampling in Experiment 2, and because their capture rate is unlikely to be improved due to low mobility (<15cm/day; Kirk 1972b), we decided not to use barrier-linked traps due to spatial constraints of shared research space. The altered sampling protocol limits our ability to predict inter-annual fluctuations, but provides useful estimates of oviposition site selection within common cultural treatments.

Adults were live-trapped, identified on-site, and released at the plot center. Sampling of adults was temporarily suspended from September 1-26 while tomato crops were harvested and fall tillage occurred in the tilled subplots. Unfortunately, this period of suspended sampling coincided with the peak activity period of *H. pensylvanicus*. Instead of sampling larvae in the fully-crossed experiment, we chose a subset of the

recently tilled subplots (4 of 16) once larval collections began in October, as larval capture was nearly zero across tilled treatments system-wide. . Due to the limited mobility of larvae (Kirk 1972b), the doubled pitfall trap density in the subset of plots was unlikely to confound our measures of larval activity density in tilled treatments. We attempted to use multiple sampling methods for larvae separately throughout the season, including soil cores, quadrats, sentinel prey, and litter-bag surveys, but only pitfall traps yielded sufficient data for analyses. Captured larvae were collected and stored in ethanol. Additionally, we harvested living weed biomass from no-till plots on July 13 using one 0.1 m^2 quadrat per plot, to examine possible associations with available plant resources. Due to the severe drought in 2012, there was very little weed growth after the July sample date, thus no further weed control occurred, and sampling was terminated. The fall weed community was largely composed of the maturing plants that were measured in July. Weed data were not analyzed in the tilled plots because weed regrowth was negligible in the autumn following tillage operations, when all the larval sampling occurred.

2.2.3   Statistical Analyses

We analyzed activity densities of *H. pensylvanicus* adults and larvae as seasonal sums per replicate plot in both experiments. The two experiments performed in 2011 and 2012 were analyzed separately, due to differing cultural treatments, collection methods and plot sizes. We performed all analyses in R version 3.0.2 (R Core Team 2013).

Experiment 1) Adult *H. pensylvanicus* pitfall trap captures were ln(x+1) transformed and compared between the four cultural weed control treatments (TILL, TILL+CLOVER,

STRIP-TILL RYE + CLOVER and NO-TILL RYE) using ANOVA, and pairwise

comparisons were made between treatments using Tukey's HSD test. Blocking variables

did not explain a significant amount of variation, and were removed from analyses.

Because seasonal larval captures in the tilled plots were almost uniformly zero counts, the

distribution did not meet the assumptions of ANOVA, and was also inappropriate for

rank-based analysis. We used random permutation tests, which are particularly useful for

small, zero-inflated datasets (Anderson 2001, Legendre and Legendre 2012). To do this,

the observed F statistic across treatments was compared to 10,000 F statistics calculated

from permuted distributions of the larval dataset across all treatments. P-values calculated

were the proportion of randomly generated F-values that were greater than the observed

F-value. Pairwise tests between treatments were made by comparing observed differences

in mean larval catch for each of the 6 possible treatment combinations to 10,000

randomized mean differences from permuted distributions. Associated P-values were

calculated as the proportion of randomly generated mean differences that were greater

than the observed differences.

Experiment 2) Adult *H. pensylvanicus* captures were ln(x+1) transformed and cover crop

treatments (fallow, mustard, rye, and rye/vetch) and the two tillage treatments were

evaluated using a two-way ANOVA, with cover crop and tillage treatments as categorical

predictor variables, blocked by main plot. Larval captures were also ln(x+1) transformed

and the five (four cover crop + 1 tilled) treatments were analyzed with a one-way

ANOVA. The tilled subplots were structurally identical, so instead of using a fully

crossed design, we added a subset of tilled plots as fifth cover treatment in analyses,

including "plot" as a random factor to avoid pseudo-replication. Block and main plot variables did not explain significant amounts of variation, and were subsequently removed from analyses. Larval and adult captures were also regressed with living weed biomass within each plot, which was also ln(x+1) transformed.

2.3   RESULTS

2.3.1   Larval activity phenology

Harpalini larvae became surface-active in late October in both experiments, 4 to 6 weeks following the peak in adult *H. pensylvanicus* activity (Figs 2a and 2b). Larvae were captured in pitfall traps on October 24 and November 9 in 2011, and on October 11, 17 and 25, November 1 and 15 in 2012. A peak in larval capture was observed during the 3rd week of October 2012, but trapping was terminated too early to determine an activity peak for 2011. Trap capture for adults in the 2012 experiment was extremely low, with only 20% of the mean nightly capture compared to the previous experiment, partially due to differences in trapping techniques and the presence of a killing agent (soapy water) in the traps in 2011.

2.3.2   Experiment 1

Seasonal activity densities for *H. pensylvanicus* adults were more than 5-fold greater ($F_{3,16}$=17.060, p<0.005; Fig. 2.3a) in the NO-TILL RYE plots and the STRIP-TILL RYE + CLOVER plots than in the cultivated treatments (TILL and TILL+CLOVER). Larval activity was only observed over two sampling periods in 2011, and was relatively low because sampling did not continue through the larval activity peak. Still, larvae showed similar trends to adults between treatments (significant treatment effect: F=2.928, n=20

p=0.040; Fig. 2.3b), except that seasonal activity density only differed between NO-TILL RYE and cultivated treatments (TILL and TILL+CLOVER; Fig 2.3b).

2.3.3 Experiment 2

Unlike Experiment 1, there were no differences in the activity density of *H. pensylvanicus* adults across tillage ($F_{1,24}$=0.301, p=0.588; Fig 4a) or cover crop treatments ($F_{3,24}$=1.142, p=0.352; Fig 2.4a). This absence of effects may have been due to the different trapping strategy employed in 2012, as well as the long gap in sampling during the *H. pensylvanicus* activity peak. Harpalini larval activity varied across treatments ($F_{4,15}$=5.395, p=0.007), and was higher in the mustard and fallow plots than tilled plots or rye/vetch (Fig 2.4b), although tilled plots did not differ from rye or rye/vetch treatments. Within the no-till treatments, larval captures positively correlated with total weed biomass (r^2=0.2654, df=13, p=0.049; Fig 2.5a), but adults did not (r^2=0.021, df=13, p=0.608; Fig 2.5b).

2.4 DISCUSSION

The treatments in Experiment 1 allowed us to examine adult and larval carabid activity over both extreme and subtle variations in tillage frequency. Like several other studies (Brust and House 1984, Cromar et al. 1999, Shearin et al. 2008), adults foraged disproportionately in treatments with reduced cultivation (Fig 2.3a), but were equally active in the NO-TILL RYE and STRIP-TILL RYE +CLOVER treatments, even though the strip-tilled plots were moderately more disturbed. Both of those treatments had high weed growth (Butler 2012), which may be an important cue seed-feeders use to identify foraging environments (Blubaugh et al 2011). In fact, the weeds and cover crops present in this plots may have hampered movement and decreased pitfall capture, thus, our

results may underestimate the true magnitude of weed growth effects on carabid assembly.

Larvae were almost completely absent in frequently tilled sites (Fig 2.3b), even though tillage operations were terminated more than two months before larval emergence was observed. Compared with the two cultivated treatments, larval activity density was higher in the least-disturbed NO-TILL RYE treatment, but not in the STRIP-TILL RYE + CLOVER treatment, suggesting that adult females have oviposition preferences for sites that have been free of cultivation for at least one growing season. Given that *Harpalus spp.* larvae have rather limited mobility on the soil surface (<15 cm radius around burrows; Kirk 1976b), locations where larvae were captured should serve as a reasonable estimate of oviposition site.

We found additional support of carabid oviposition preference for less disturbed crop environments in Experiment 2. Although larvae have been observed before in cultivated fields (Kirk 1976a, Holland et al 2007), our lowest larval captures were in tilled plots. *H. pensylvanicus* overwinters in the vulnerable larval stage, and may be limited by perennial, thatch-insulated overwintering habitat and dispersal ability (Hof and Bright 2010, Fox et al. 2013). With this in mind, it was surprising that we observed lower activity densities in the rye and rye/vetch plots (Fig 4b), as those treatments were the only ones with undecomposed cover crop residue remaining in late autumn. Lundgren et al (2013) found contrasting results; almost 3x as many carabid larvae were captured in plots with a killed rye cover crop compared with plots treated with herbicides, although larvae were not identified beyond the family level. In that particular study, weed growth was more than twice as high in the rye-mulch plots, and this may be a key variable that

predicts larval density. It could explain the absence of larvae in our rye and rye/vetch

plots, where weed growth was much lower than in all other treatments (Fig 2.5). In fact,

weed biomass positively correlated with larval activity in no-till plots (Fig 2.5a), and

Holland et al (2004) found a similar spatial association of carabid larvae with weed

cover, although larvae were not specifically identified. Previously documented seed-

caching behavior by *Harpalus* larvae (Kirk 1972b, Hartke et al 1998) suggests that weeds

are a critical food resource, perhaps more important for oviposition site selection than

insulating thatch.

Alternatively, low capture rates in the rye and rye/vetch plots could be due to the

limited mobility of larvae and the structural impediment formed by the dense thatch

layer. For this reason, pitfall trap captures may be a poor estimate of foraging activity in

heavy soil-surface vegetation (Greenslade 1964, Thomas et al. 2006). Improved sampling

strategies must be developed to more accurately describe spatial distributions of carabid

larvae in heterogeneous habitats, particularly those species that are less surface active.

While pitfall traps were deployed, we tried several additional sampling methods to

estimate larval density, including soil cores, quadrats, and even plot excavation; these

yielded very few larvae. Soil emergence traps may prove much more useful for

estimating larval survival and density (Holland and Reynolds 2003, Holland et al 2007),

but because they require almost a full year without disturbance to document overwinter

survival for fall-breeding species, they are difficult to implement in working crop

systems.

The differing pitfall trapping methods employed between years precludes

quantitative analysis of inter-annual variation in captures, but system-wide, we found

very few *H. pensylvanicus* adults in Experiment 2 compared with Experiment 1. This could be related to the fact that the crop areas, margins and adjacent fields were all cultivated in spring 2012 after 10 fallow years, destroying the nearest source populations of fall-active carabids. The low adult capture rates could also be explained by the absence of the barrier link between traps, or the fact that much of the peak activity period for *H. pensylvanicus* adults was missed during the 2012 sampling periods.

Unlike Experiment 1, we found no differences in activity density of *H. pensylvanicus* adults between cover crop types, nor between tillage treatments in Experiment 2 (Fig 2.4a). Treatment effects consistent with those observed previously may have been less detectable due to the extremely low adult capture system-wide. Still, the cultivated treatments were substantially different from those in Experiment 1 because cultivation occurred only twice, once in the spring shortly before crop planting, and again in September. Ward et al. (2011) found a similar absence of effects due to infrequent tillage with adult *H. pensylvanicus* foraging in sweet corn. Sparse weed cover and longer disturbance intervals may provide adult beetles with adequate foraging habitat (Shearin et al. 2007), but requirements may be more stringent when selecting safe overwintering and oviposition sites.

In both experiments, we found that activity densities of larvae were not consistent with habitat use by adults; adults actively foraged in sites with infrequent (annual) tillage (Figs 2.3a and 2.4a), but larvae were rarely captured in sites with soil disturbance, even when it occurred as much as six months prior (Fig 2.3b). This habitat-use/oviposition site discrepancy is important to note because nearly all the work done to quantify carabid communities and their ecosystem services in crop environments focuses on the activity

density of adults (Kromp 1999). This may not accurately describe the ecological costs of cultivation practices for carabid communities, and emphasizes the importance of perennial non-crop habitat in agricultural landscapes. Adults readily colonize crop areas within weeks following tillage (Varchola and Dunn 2001, Ward et al 2011), but suitable oviposition sites require a much longer disturbance interval. Across all life stages, carabids common in agricultural systems may be less resilient to seasonal disturbance than assumed (Holland and Luff 2000, Jonason et al. 2013).

Recommendations for enhancing weed seed predation services on-farm are to delay tillage until late in the fall or spring (Menalled 2008, Ward et al. 2008). This strategy maximizes the time weed seeds are exposed to soil-surface seed predators, but does not consider oviposition preference of invertebrate granivores. Delaying tillage may provide an optimal egg laying site due to enhanced weed cover and food resources in the fall when many carabids breed (Lovei and Sunderland 1996), and a substantial population of larvae may be compromised when tillage occurs the following spring (Purvis and Fadl 2002). Our data suggest that while *H. pensylvanicus* are strong dispersers as adults, they very rarely oviposit in disturbed habitat. Undisturbed soil and living vegetation are critical components of oviposition site quality, but non-crop habitat is increasingly rare in the large-scale agricultural landscapes of the US Midwest. Incorporating perennial refuge space or strip-tillage practices that reduce soil disturbance between crop rows may improve safe-site limitations for overwintering larvae, and promote ecosystem services by following generations of weed seed predators.

Acknowledgements

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Figure 2.1 Third-instar (*Harpalus sp*.) collected from experimental field plots in Tippecanoe County Indiana, USA

Figure 2.2 Raw means ±SE nightly pitfall trap capture of adult *Harpalus pensylvanicus* individuals (solid line) and Harpalini larvae (dotted line) from no-till treatments in separate experiments performed in a) 2011 and b) 2012 in Tippecanoe County, Indiana, USA
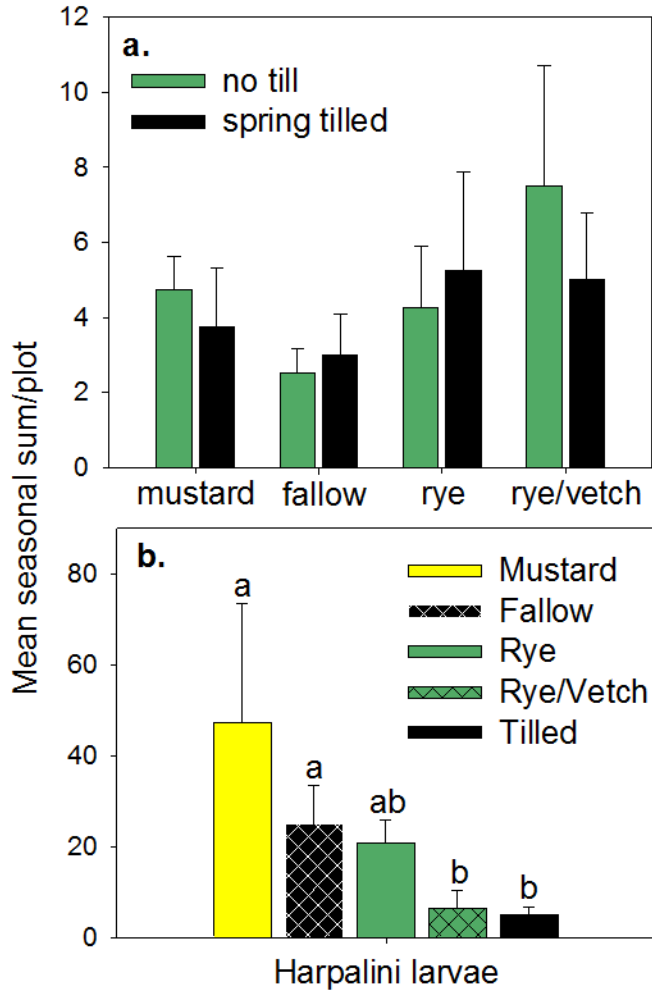
Figure 2.3 Seasonal sums (means + SE) of a) Harpalus pensylvanicus adults and b) Harpalini larvae captured in pitfall traps between rows of tomatoes transplanted into NO-TILL RYE, STRIP-TILLED RYE, TILL+CLOVER, and TILL treatments. Significant differences between groups are labeled with different letters
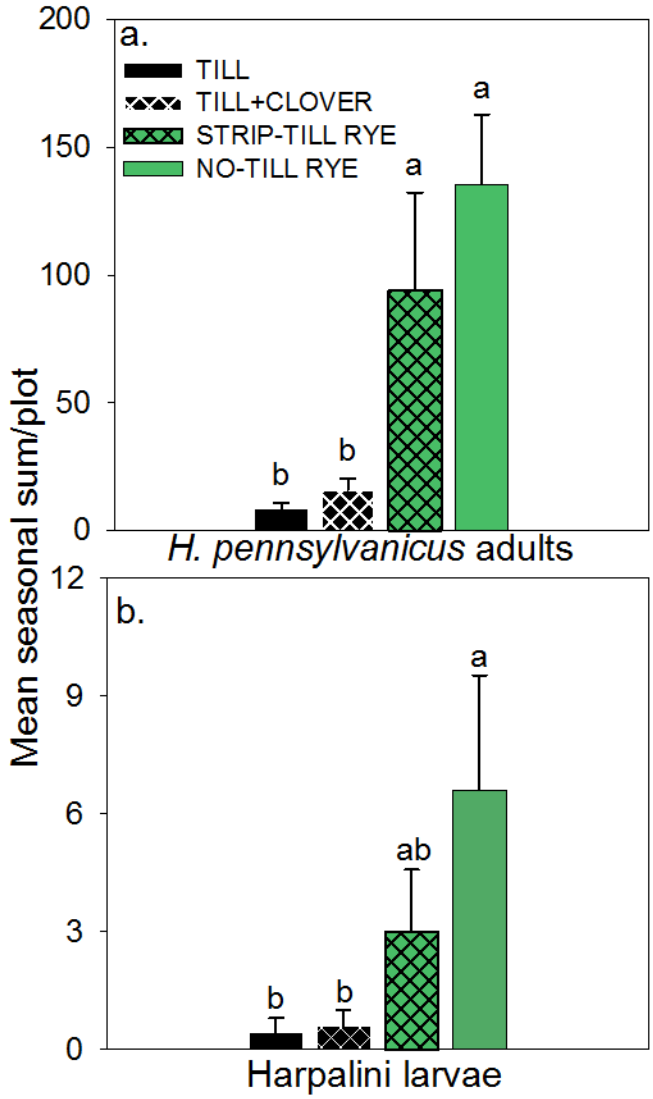
Figure 2.4 Raw means + SE 2012 seasonal sums of a) *Harpalus* adults and b) Harpalini larvae captured in dry pitfall traps between rows of tomatoes transplanted into black plastic mulch. Significant differences between groups are labeled with different letters
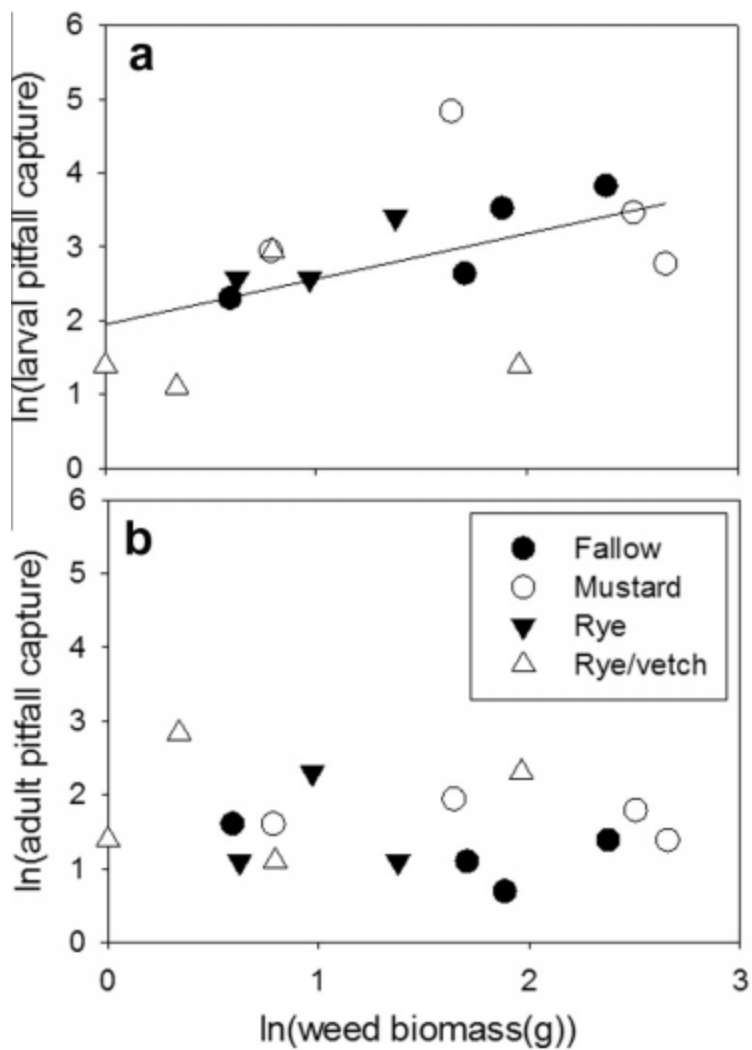
Figure 2.5 Regression of total weed biomass samples harvested in July 2012 on seasonal 2012 sums of a) Harpalini larvae and b) *H. pensylvanicus* adults captured in pitfall traps within the no-till treatments. Both axes are plotted on a natural logarithmic scale.
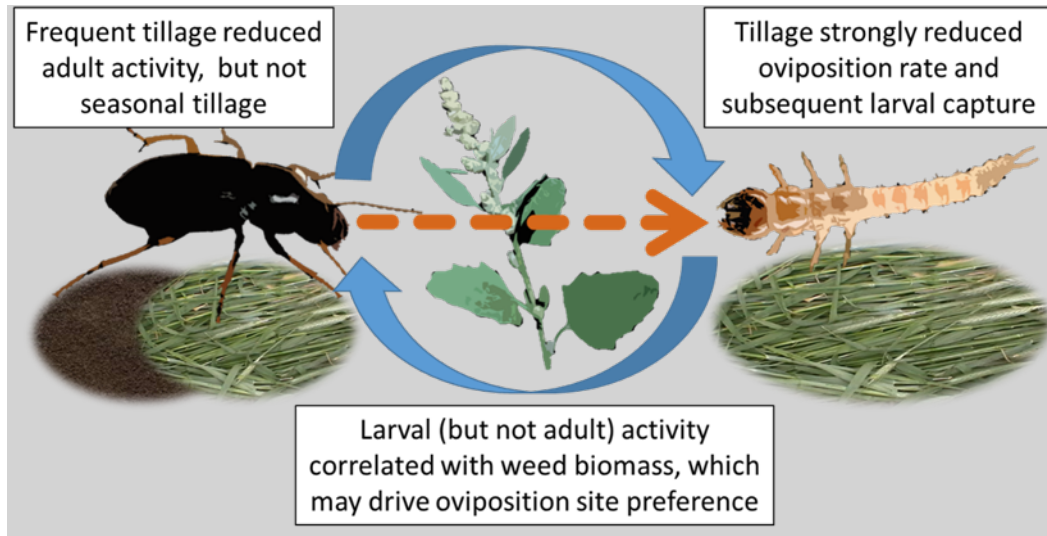
Figure 2.6 Graphical abstract

CHAPTER 3.   COVER CROPS PROMOTE AGGREGATION OF
OMNIVOROUS PREDATORS IN SEED PATCHES AND FACILITATE
BIOLOGICAL CONTROL

Running head: Quantifying omnivore feeding behavior using gut content analysis

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3.1   ABSTRACT

Omnivores are important conservation biological control agents of both weed seeds and

insect pests, and cultural strategies, like cover crops, are suggested to promote their

ecosystem services. However, few studies establish direct links between cover, food

resources, and pest suppression, because basic biological resources are entangled and co-

occurring in working agroecosystems, and direct documentation of predation events is

difficult. Here, we use immuno-marking tools to analyze gut contents and examine both

aggregation and consumption by omnivorous predators in response to experimentally

manipulated vegetative cover, weed seeds and invertebrate prey. In the three common

ground beetle species studied, we found evidence of seed feeding in 19% of beetles assayed, including some species considered largely predaceous. Only 8 % tested positive for prey. Early in the season, seed resources increased capture of omnivorous ground beetles by 77 %, but prey had no effect, suggesting that they track reliable food resources at lower trophic levels. This response to seed resources disappeared by autumn in both years of the study. While aggregation patterns around food and habitat resources were species and context-specific, gut content analysis revealed that vegetative cover facilitates consumption of seeds, increasing seed predation frequency by 73% across all species examined. Cover had no impact on consumption frequencies of prey, but seed availability reduced prey consumption by 27%. These results imply that the microclimate and biological resources associated with vegetated habitat may promote biological control via increased omnivore activity density, but only plant-based foods induce predictable effects on foraging behavior.

Keywords: Carabidae, immunomarking, gut content analysis, omnivory, seed predation, weed biological control

3.2   INTRODUCTION

Omnivory is pervasive among generalist natural enemies in agricultural systems where broad diet breadth enables predators to persist despite seasonal disturbance and food insecurity (Eubanks and Denno 1999). For this reason, omnivores are critical to biological control (Ågren et al. 2012), preventing pest outbreaks by consuming pests at low densities before carnivores assemble (Eubanks 2005). Plant-based food resources can confer stronger top-down suppression by reducing emigration and supporting higher densities of omnivorous predators, despite reductions in per-capita prey consumption due

to plant feeding (Eubanks and Denno 2000, Eubanks and Styrsky 2005, Maselou 2014). In addition to prey and plant-based food, structural habitat resources also determine omnivore retention and feeding behavior by providing shelter and refuge from predation (Landis et al. 2000, Kratina et al. 2012). However, the ways that food and habitat resources complement and interact with each other are unknown because each are difficult to isolate in the field.

Omnivores pervade nearly every genus of the Carabidae, and because their feeding ecology is well known at the species level (Lundgren 2009), carabids make excellent subjects for evaluating how food and habitat resources drive predator assembly. They are important predators of invertebrate pests (Lundgren and Fergen 2011), and weed seeds (Menalled et al. 2007), and several studies have examined omnivorous carabid assembly around seed or prey resources. Most report no numerical response by carabids to seed density (Marino et al. 2005, Westerman et al. 2008, Baraibar et al. 2012; but see Frank et al. 2011), or prey density (Birkhofer et al. 2007, Frank et al. 2011, Hassan et al. 2012). However, carabids commonly aggregate around plant resources (Brooks et al. 2012, Diehl et al. 2012, Blubaugh and Kaplan 2015), and vegetative cover may be a missing link that facilitates a numerical response to food availability.

The field is rich with correlative evidence of omnivorous carabids' associations with vegetative cover (Carmona and Landis 1999, Shearin et al. 2008, Diehl et al. 2012), but few studies take a mechanistic approach to understanding how basic environmental resources shape activity patterns of biological control agents. In working agroecosystems, cover and food resources are entangled and co-occurring, making it difficult to predict their respective effects on biological control. Further, because most studies are limited by

reliance on indirect sampling methods, direct links between predator activity and pest suppression are rare (Griffiths et al. 2008). Cover supports higher densities of natural enemies by providing an optimal microclimate, and also through provisions of non-pest food resources associated with vegetated habitats (Diehl et al. 2012), which may distract biological control agents from pest suppression (Frank et al. 2010). Without directly measuring pest predation events by omnivores, we cannot confirm that increased natural enemy activity confers biological control.

Modern molecular tools make direct measurements of trophic interactions more tractable (Symondson and Harwood 2014), and the development of affordable immuno-marking techniques enable efficient identification of predator gut contents at the field scale (Hagler 2006, 2011, Lundgren et al 2013, Kelly et al 2014). Vertebrate immunoglobulin proteins can be easily incorporated in insect food resources or on prey items, providing a persistent, reliable, specific mark in the guts of insects (Hagler 1997b), detectable with IgG specific ELISAs (enzyme-linked immunosorbent assays). In this experiment, we manipulate and disentangle three common biological resources: vegetative cover, seeds, and invertebrate prey. We use protein-based marking techniques to link consumption frequencies of both seeds and prey with foraging activity of three numerically dominant carabid species in agroecosystems. Diet mixing can have a synergistic effect on predator fitness (Eubanks and Styrsky 2005), thus we predicted that availability of both seeds and prey resources would promote higher carabid activity densities than either food resource alone. We also predicted that cover would provide an optimal foraging microclimate for natural enemies, facilitating predator activity and consumption of both insect prey and weed seeds.

3.3   METHODS

3.3.1   Study system

This experiment was conducted at the Purdue University Meigs Horticulture Research

Farm near Lafayette, Indiana, USA over the 2012 and 2013 growing seasons. In March

2012, forty 3m^2 plots were tilled with 2 m buffers between each. Resource manipulation

treatments were applied in a 2x2x2 factorial design, in 5 randomized blocks. The

combinations of three variables—presence/absence of plant cover, presence/absence of

seed resources, and presence/absence of prey resources—comprised 8 treatment groups.

Experimental plots were located in an agronomic crop landscape, surrounded on two

sides by a grassy margin, and two sides by conventionally managed soybeans. The cover

treatment was established by drill-seeding rhizobium-inoculated red clover (*Trifolium*

pretense L.) at a depth of 0.5 cm, at a rate of 13 kg/ha in half the plots. Bare plots and the

margins were treated with pre and post-emergent herbicides (a mixture of oryzalin,

simazine and glyphosate) in May and August in 2012 and 2013, to maintain a bare-soil

environment without tillage. These chemicals are neither toxic nor repellant to adult

carabids (Brust 1990). To control available seed resources, clover plots were mowed and

weeded as needed to prevent seed rain.

Once the cover crop treatment was established, common lambsquarter

(*Chenopodium album)* seeds (purchased from Azlin Seed service, Leland, Mississippi,

USA) were dispersed evenly with a parmesan cheese shaker while walking a grid. Seed

subsidies were applied at a rate of 15,000 seeds/m^2, a moderate weed seed rain density

(Davis and Raghu 2010). Frozen *Drosophila melanogaster* pupae (purchased from Spider

Pharm, Yarnell, Arizona, USA) were added to prey resource treatment plots at a density

of 10,000 pupae/m^2, matching the seed treatment by weight. Because this was >10x

higher than documented ambient densities, the application rate was reduced to 850

pupae/m^2 (per Frank et al. 2011) after the first subsidy in June 2012, and for all

subsequent applications. *C. album* seeds and *D. melanogaster* pupae were selected as

food resources because they are both palatable to ground beetles, similar in size, and

common in the agricultural environment where the experiment occurred (C. Blubaugh

personal observation). *C. album* is a weed of great economic importance, and useful focal

species for measuring weed seed biological control. *D. melanogaster* is not a pest, but

serves as an adequate target to model natural enemy responses to immobile pests (in egg,

larval, or pupal form) on the soil surface.

Before deployment in the field, seeds were marked with rabbit IgG and pupae

were marked with chicken IgY. The vertebrate proteins were purchased as lyophilized

powder (Equitech Bio, Kerrville, TX), and dissolved in 1mg/mL solution, which was

sprayed on the respective foods at a rate of 3mL/10g of food material. Seeds and pupae

were fully air-dried before they were broadcast in the field. Food subsidies were applied

in 2012 on June 7, August 9, and September 9 and in 2013 on May 23, June 9, July 7,

August 20, September 3 and September 19.

3.3.2   Field sampling

We measured carabid activity density using pitfall traps, which consisted of two 950 mL

deli cups in each plot, sunk in the ground flush with the soil surface, connected by a 0.2

m tall barrier made of 1 m aluminum flashing. To preserve the internal protein marks in

carabids' guts, traps were dry with no liquid killing agent, and 1 cm of grass clippings in

the bottom provided refuge for trapped animals, reducing the likelihood of cross-

contamination of the protein mark due to predation events in the trap (King et al. 2008). Traps were set at dusk on nights without precipitation and collected at 09:00 each morning. Daily collections continued for 14 days following deployment of labeled food resource subsidies, and weekly thereafter. Trapping was suspended for 2-3 weeks each year in mid-summer, when carabid activity levels are temporarily depressed due to high nightly temperatures (Lovei and Sunderland 1996). Trapped insects were transported to the lab, identified, immediately transferred to 1.5 mL centrifuge tubes and frozen at -25 degrees C.

3.3.3   Gut content analysis

Protein markers are detectable under field conditions for a limited period of time, thus only 3865 out of 6766 beetles captured in the field experiment were used for gut content analysis. The rest were retained as a reference collection. Rabbit IgG is detected reliably under field conditions for up to 14 days, while chicken IgY is detectable for only 7 days (Hagler 1997), therefore only beetles captured within the reliable window of detection for each protein marker were tested using ELISA. To provide a definite link between the protein marker and an actual predation event (rather than external physical exposure), we dissected guts prior to analysis on all predators greater than 1 cm in length (per Lundgren et al. 2013). Indirect sandwich ELISAs were performed according to Hagler 1997. Briefly, we homogenized samples in tris-buffered saline solution, coated plates with a primary antibody (anti-rabbit IgG from goat or anti-chicken IgY from rabbit; Sigma-Aldrich, St. Louis, MO, USA), and incubated them overnight. Then we applied a 1% milk blocking agent, followed by aliquots of the macerated predator gut samples. Plates were washed with phosphate-buffered saline with tween, secondary antibodies (anti-

rabbit or chicken conjugated with horseradish peroxidase) were applied, and then plates were washed again. Finally, substrate (TMB solution) was added, and after 10 minutes optical density (OD) was measured by a microplate reader at 650nm. Our positive mark OD threshold was 3 standard deviations above the mean of 8 negative control samples which were included on each plate.

3.3.4 Protein mark retention trial

We used *Harpalus pensylvanicus* DeGeer, the most common beetle in our system, as a model for a protein mark retention experiment. Beetles were starved for 24 hours, then fed ad libitum for 24 hours on either marked seeds or pupae, each in a plastic 100 mL Solo™ cup with moistened filter paper. After 24 hours, beetles were removed and either starved or fed unmarked diet. To identify a reliable mark retention window, beetles were removed in groups of 8 and frozen for dissection and gut content analysis after 0, 12, 24, 48, 72, and 96 hours.

3.3.5 Statistical analysis

Activity density of carabids was analyzed with mixed-effect, quasi-poisson generalized linear models (to handle overdispersion) for each of the three most dominant species, using the glmmPQL function in the MASS package of R. The response variable was nightly pitfall capture, and fixed effects were cover, seed availability, and prey availability. We included a fixed effect of 'season' for our analysis of *H. pensylvanicus* activity density, because it exhibits two annual activity peaks. The first occurs during mid-summer after 2nd-year adults emerge from overwintering habitat, and the next occurs after a brief summer aestivation when pupating larvae emerge during breeding season

(Kirk 1973). Random effects for activity density models were 'plot' nested in 'block', and 'Julian day' nested in 'year'.

Gut content results were analyzed using mixed-effect GLMs for each protein marker (seed or prey), assuming binomial distributions. The response variable was the proportion of insects testing positive for the respective food resource, calculated from seasonal sums of all three carabid species per plot. We pooled species and sample dates for this model for an annual estimate of consumption frequency and biological control services performed by the carabid community across resource manipulations. Also, for each food marker, we restricted the analysis to plots that contained subsidy treatments of that particular food resource (seed or prey), enabling us to examine how alternative food alters foraging behavior on the focal food resource. Fixed effects were cover and either seed or prey; random effects were 'plot' nested in 'block'.

3.4   RESULTS

3.4.1   Pitfall sampling

We captured 6766 carabids over two years, >85% of which were three numerically dominant species: *Anisodactylus sanctaecrucis* Fab*, Poecilus chalcites* Say, or *H. pensylvanicus. A. sanctaecrucis* and *P. chalcites* are both spring-breeding species, which go through larval stadia during the summer and overwinter as adults. *H. pensylvanicus* comprised more than half the annual trap catch, and it is a fall-breeding species, overwintering in the larval state (Fig 3.1).

P.chalcites was almost twice as active in plots with cover crops (Fig 3.2a, Table 3.1), but did not respond to either of the food resource subsidies. *A. sanctaecrucis* aggregated around weed seeds in both the cover and bare ground treatments, and cover

crops marginally reduced its activity density (Fig 3.2b, Table 3.1). *H. pensylvanicus* had a synergistic response to weed seeds and cover early in the season (significant seed*cover*season interaction; Fig 3.2c, Table 3.1), but this relationship disappeared later in the fall during the second activity peak (Fig 3.1), after which only the cover treatment promoted activity density. None of the carabid species demonstrated any response in the field to fly pupal subsidies (not pictured).

3.4.2   Gut content analysis

Mark retention of both rabbit IgG and chicken IgY was reliable up to 72 hours after feeding events in *H. pensylvanicus* (>80% of individuals tested positive; Fig 3.3), and the mark decay rate did not differ between beetles that were starved after consuming labeled food and those that fed on unmarked diet (pooled data shown).

Among the species examined, *H. pensylvanicus*, a known seed predator (White et al. 2007), was the most frequent consumer of seeds (Fig 3.4). *P. chalcites* is often considered a strict predator (Lund and Turpin 1977, O'Rourke et al. 2006), and was the most frequent consumer of pupae, but tested positive for seeds almost as frequently. *A. sanctaecrucis* had very low positive mark rates in general, and it consumed seeds more often than prey (Fig 3.4).

Across all three carabid species and sample dates, the cover treatment increased the likelihood of consuming seed resources by 50%, and while showing a negative trend, availability of fly pupae did not significantly reduce seed predation frequency (Fig 3.5, Table 3.2). The effects observed were largely driven by *H. pensylvanicus,* which made up more than 75% of the beetles that tested positive for seed material. Rates of pupal consumption were only half of those observed for seed consumption (Fig 3.5b).

Proportions of beetles consuming pupae in plots with pupal subsidies was marginally reduced by seed availability, but unaffected by cover (Fig 3.5b, Table 3.2).

3.5   DISCUSSION

This work demonstrates clear behavioral and trophic links between omnivorous natural enemies and plant-based food (i.e. seeds). Omnivores (*A. sanctaecrucis* and *H. pensylvanicus*) assembled in seed patches (Fig 3.2) and frequently consumed them (Fig 3.4). Even *P. chalcites,* which is a reluctant seed-feeder in laboratory trials (Lund and Turpin 1977, O'Rourke et al. 2006), occasionally consumed seeds. Lundgren et al. (2013) also discovered many unlikely seed consumers using gut content analysis, suggesting that seed-feeding and omnivory in general are even more ubiquitous than previously considered. Omnivorous predators frequently select lower-quality food sources that are stable and abundant (Denno and Fagan 2003), and weed seed resources are almost perpetually exploitable in agroecosystems. Selection on omnivores in this system seems to promote tracking food resources at lower trophic levels (sensu Eubanks and Denno 2000, Frank et al. 2011).

None of the omnivores we observed responded to prey resource availability (Fig 3.2), not even the mostly-carnivorous *P. chalcites*, which consumed pupae more often than the other carabids (Fig 3.4), in contrast to predictions. This result is consistent with Frank et al. (2011), but differs from Brooks et al. (2012), who discovered stronger links between predators and invertebrate prey availability. None of the variables examined had any impact on prey consumption (Fig 3.5b). In general, consumption of prey resources in our system was very low, relative to seed consumption (Fig 3.5a). Weak responses to prey subsidies could be related to the lower densities at which they were applied relative

to seeds, but our manipulated treatments approximated naturally-occurring densities. Thus, plant and prey resources appear not to evoke a synergistic response by omnivorous predators in our system.

While both omnivorous carabid species tracked and consumed weed seeds, they seem to have differing spatial niches. Specifically, *A. sanctaecrucis* foraged equally in both exposed and cover crop habitat (Fig 3.2a), and *H. pensylvanicus* was more active in cover crop habitat (Fig 3.2c). Because *A. sanctaecrucis* is active early in the season, its life history may be linked to winter-annual weeds, which often germinate in exposed environments following fall tillage operations in temperate agronomic systems (Brooks et al. 2012). Being fall-active *H. pensylvanicus* is linked with summer annual weeds, which senesce in autumn and accumulate more biomass than winter-annuals. Niche complementarity that results from contrasting habitat selection among weed seed predators will capitalize their ecosystem services, as agrocosystems are typically a complex matrix of tilled and vegetated habitat patches.

The early season synergistic response of the most common carabid in our system, *H. pensylvanicus,* to seeds and cover was particularly interesting because it suggests that provisions of vegetative cover can indeed promote seed patch depletion by *H. pensylvanicus*, which is the dominant seed predator species in many North American cropping systems (Ward et al. 2014, Blubaugh and Kaplan 2015). By autumn, the synergistic response of *H. pensylvanicus* to seed and cover resources dissolved (Fig 3.2c). High activity densities of *H. penylvanicus* during the peak activity period may have caused interference competition between beetles in seed patches, promoting dispersal to

all available vegetated habitats, overwhelming the preference for seed patches demonstrated early in the season.

Gut content analysis revealed that cover crops facilitated seed-foraging behavior across the entire season and all three species. In plots that contained seeds, a direct consumption event was twice as likely if red clover was present (Fig 3.5a). Alternative prey availability (pupae) did not significantly reduce seed consumption frequencies, suggesting that omnivores' trophic links to seeds are stable and predictable in our system. Very few studies measure direct links between seed predator activity and predation events in the field via predator observation (Brust and House 1988), or gut content analysis (Lundgren et al. 2013). Numerous studies make indirect, episodic estimates of weed seed predation in varying structural habitats (reviewed in Meiss et al. 2010), but these are limited by their uncertainty of seed fate after removal (VanderWall et al. 2005). This research implicates living plant biomass as an inducer of seed consumption, validating the utility of cover crops to promote weed seed predation services by increasing seed predator activity density as well as increasing per-capita predation frequency.

In summary, our results suggest that omnivores track weed seed resources, but not prey resources in the field, and strict predators were not able to identify either type of resource patch. For this reason, omnivorous carabids seem most competent as conservation biological control agents of weed seeds. Weeds are persistent problem in both horticultural and agronomic systems, even with widespread adoption of herbicide tolerant crops. As weed species evolve resistance to multiple modes of herbicide action, a return to integrated management tools will be necessary (Davis et al. 2012). Seed

predation can be an important component of a multi-faceted approach that can reduce propagule pressure (Westerman et al. 2006) and slow the spread of resistant weed populations (Mortensen et al. 2012). Our research shows that even when alternative prey are available, carabid seed predators have the ability to identify and preferentially forage in seed patches. This work provides powerful evidence that vegetative cover not only provides optimal microclimate for natural enemies (Saska et al. 2010), but directly facilitate seed consumption. Thus, provisions of cover (e.g. cover crops and forage crops) can promote ecosystem services by weed seed predators, in addition to the numerous other benefits they provide.

<div style="text-align:center">3.6   REFERENCES</div>

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Table 3.1 Results of each decomposed species-specific mixed effect GLM on nightly pitfall trap capture in plots where cover, seeds and prey were manipulated

Factor	Coefficient	SE	t	P	
a) Anisodactylus sanctaecrucis					
Intercept	-0.286	0.204	-1.403	0.160	
Seeds	0.498	0.143	3.472	<0.001	**
Cover	-0.277	0.147	-1.875	0.069	.
b) Poecilus chaclites					
Intercept	-1.233	0.370	-3.328	0.001	**
Cover	0.653	0.208	3.140	0.004	**
c) Harpalus pensylvanicus					
Intercept	-1.106	0.217	-5.09	<0.001	**
Cover	0.286	0.282	1.014	0.310	
Seeds	0.221	0.287	0.770	0.441	
Season	1.192	0.217	5.473	<0.001	**
Cover*seeds	0.589	0.364	1.615	0.106	
Cover*season	0.352	0.286	1.230	0.218	
Seeds*season	-0.187	0.294	-0.637	0.523	
Cover*seeds*season	-0.722	0.371	-1.946	0.051	*

Table 3.2 Results of mixed effect GLMs on seasonal proportions of beetles testing positive for a) seeds and b) prey, pooled across carabid species

Factor	Coefficient	SE	t	P	
a) Seeds (rabbit IgG)					
Intercept	-1.311	0.246	-5.314	<0.001	**
Cover	0.727	0.190	3.813	0.002	**
Prey	-0.302	0.1883	-1.605	0.132	
b) Prey (chicken IgY)					
Intercept	-1.518	0.206	-7.363	<0.001	**
Cover	-0.432	0.267	-1.617	0.130	
Seeds	-0.481	0.268	-1.794	0.096	.
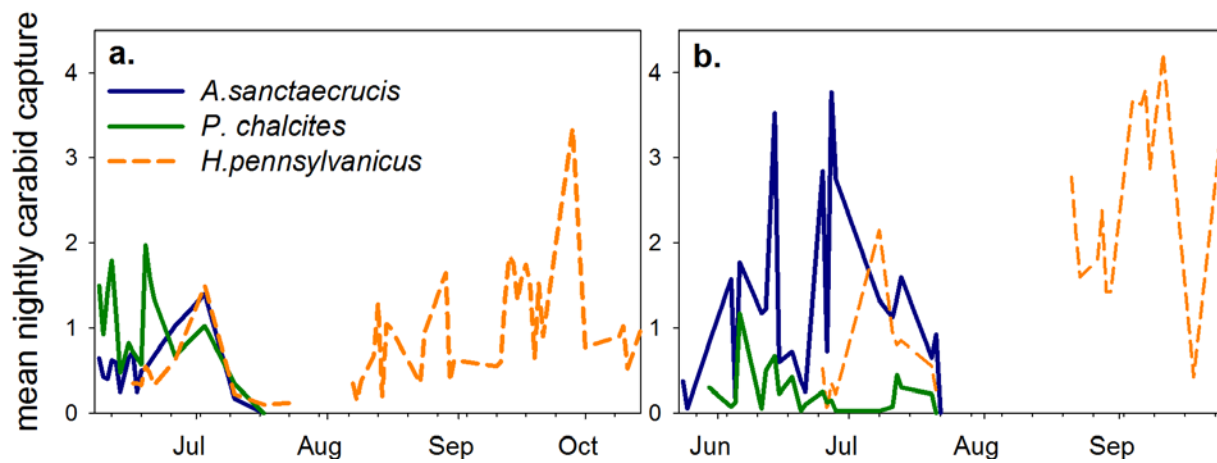
Figure 3.1 Seasonal phenology of three carabid species examined in a) 2012 and b) 2013
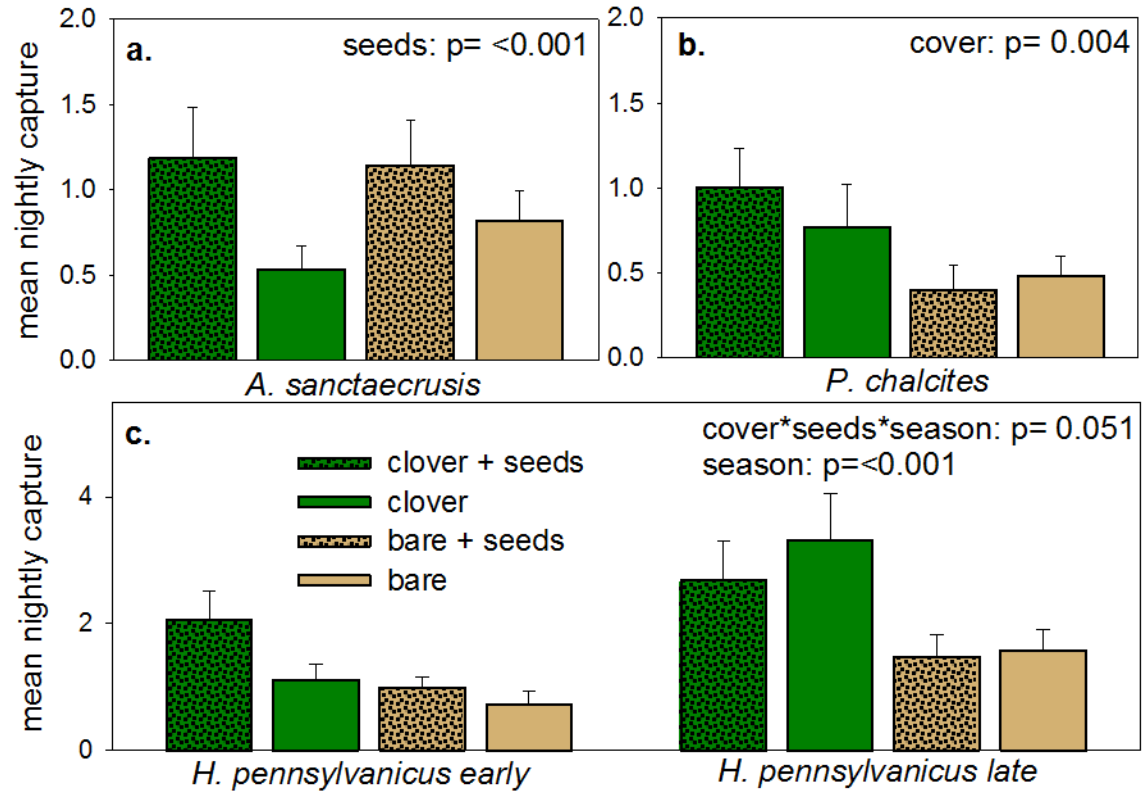
Figure 3.2. Mean (+ SE) Nightly pitfall trap capture of a) *Anisodactylus sanctaecrucis* (omnivore), b) *Poecilus chalcites* (predator), and c) *Harpalus pensylvanicus* (omnivore) across cover and seed subsidy treatments. P-values are given for the most complex significant effects and interactions.
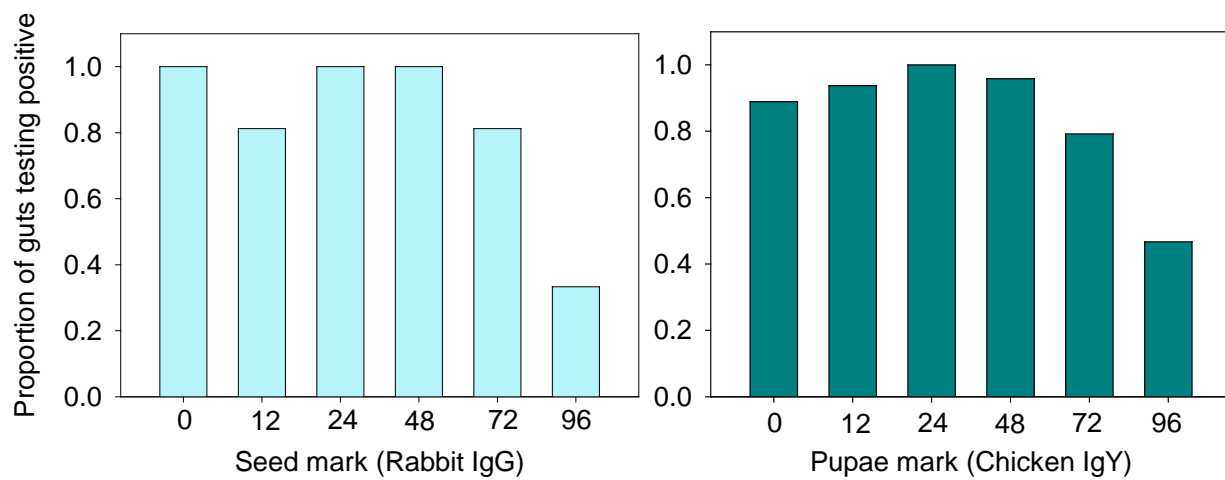
Figure 3.3 Proportion of carabids from each species assayed with ELISA which tested positive for seed- and prey-specific protein labels.
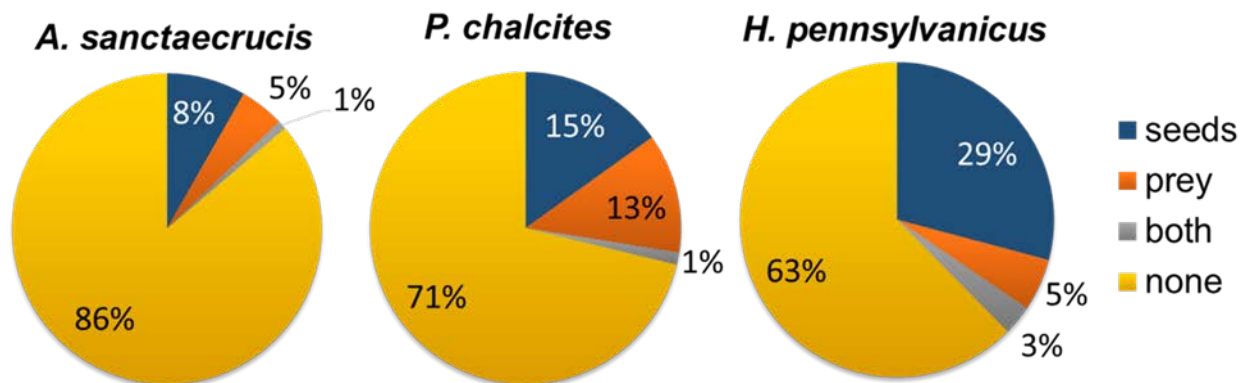
Figure 3.4 Proportion of carabids from each species assayed with ELISA which tested positive for seed- and prey-specific protein labels.
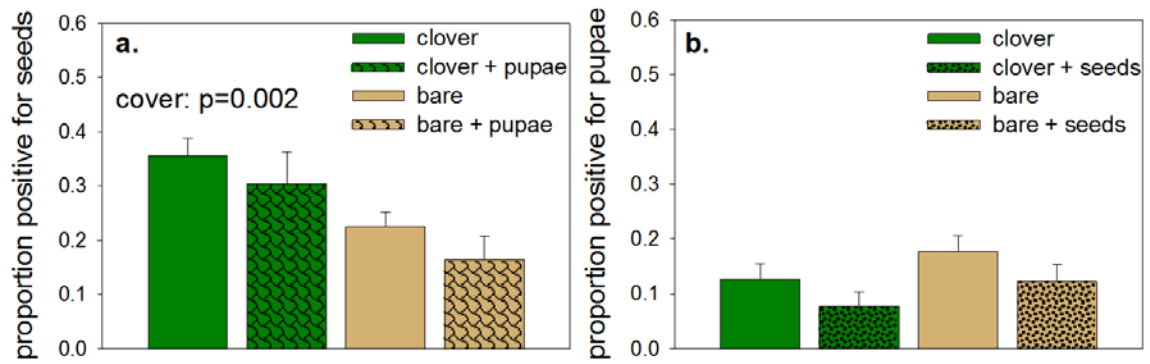
Figure 3.5 Mean (+SE) proportions of carabids testing positive for a) seeds and b) prey in gut content analysis across cover and a) prey or b) seed resource treatments. Proportions are pooled across sample dates and species, and calculated from plots that contained the focal food resource. P-values are given for significant effects.

Figure 3.6 Photo of the experimental plot matrix

CHAPTER 4.   DOES FEAR BEGET FEAR? MOONLIGHT AND HABITAT
COMPLEXITY MEDIATE INTRAGUILD PREDATION AND
NONCONSUMPTIVE EFFECTS OVER FOUR TROPHIC LEVELS

Running head: Predator avoidance cascades in food webs

4.1   ABSTRACT

Intraguild predation and predator avoidance are ubiquitous yet enigmatic drivers of food web complexity. Both processes function simultaneously, and each are mediated by refuge use in heterogeneous habitats, but their integrated impacts on top-down pressure are poorly understood. Focusing on two common seed predator taxa, mice (*Peromyscus* spp.) and carabid beetles (Coleoptera: Carabidae), we quantify cascading effects of predator avoidance on the fate of common lambsquarters (*Chenopodium album*). Mice are opportunistic insectivores and commonly prey on carabids as well as seeds, thus they act as intraguild predators even while avoiding predation from higher trophic levels. We manipulated refuge habitat availability, co-occurrence of vertebrates and invertebrate seed predators, and moonlight, which small mammals use as an indirect cue of predation risk by nocturnal avian predators. We found that avoidance of top predators by mice in both artificial and natural moonlight reduced carabid activity density in refuge habitats by up to 50%, but had weak effects on seed predation. We examined potential behavioral mechanisms using carabid foraging assays in enclosed arenas, and found that exposure to both indirect and direct vertebrate predator cues reduced their movement by 50%. In sharp contrast to our predictions, predation risk increased carabid seed consumption by

43%. Weak effects of intraguild predators on net seed removal in the field may be partially explained by compensatory seed feeding by beetles in response to predation risk, as well as seed consumption by mice themselves. This work underscores how cascading, interactive impacts of intraguild predation and predator avoidance over multiple trophic levels can influence top-down pressure on basal resources.

Keywords: Intraguild predation, non-consumptive effects, refuge, Coleoptera:Carabidae*, Peromyscus* spp.

4.2   INTRODUCTION

In recent decades, the field of trophic ecology has matured from simplistic, top-down models (e.g. Hairston et al. 1960) into complex networks driven by intraguild predation (IGP; Polis and Stron 1996), and non-consumptive effects (NCEs) of predators (Sherriff and Thaler 2014). IGP occurs when one omnivorous species preys on another which shares a common food resource (Polis and Holt 1992). NCEs are the result of prey behavioral shifts in response to perceived risk (e.g. refuge use, reduced foraging, etc; Schmitz et al. 2004). IGP can dampen trophic cascades (Finke and Denno 2004), while NCEs often exert as much top-down pressure as much as predation itself (Preisser et al. 2005). However, we still have poor predictive power over how predator-prey interactions drive community ecology because while IGP and NCEs are both ubiquitous, they are rarely evaluated together (Prasad and Snyder 2006, Frago and Godfray 2014). Simultaneously examining these interacting processes is critical because they are necessarily linked-- intraguild predators induce anti-predator responses by intraguild prey (Walzer and Schausberger 2013), influencing food webs at multiple trophic levels (Hill and Heck 2014).

Due to the logistical challenge of quantifying complex food webs (Schmitz 2006), most experiments can manageably examine only two or three trophic levels. Still, pressure from a fourth-level predator can reverse the direction of a trophic cascade (Knight et al. 2005, Grinath et al. 2014), and this limits the ecological relevance of most experimental frameworks. Seed predator networks are a useful and largely unexplored system for examining broad impacts of multi-trophic, non-linear predator-prey interactions. To determine cascading impacts of IGP and NCEs, we used seed a simple system composed of intraguild predators: nocturnal rodents (*Peromyscus spp.*), intraguild prey: carabid beetles (Coleoptera: Carabidae), and measured their combined and singular effects on a disturbance-associated broadleaf plant, common lambsquarters (*Chenopodium album* L.).

Nocturnal rodents and avian predators compose a classic study system for examining risk avoidance in trophic ecology (reviewed in Brown and Kotler 2004). From this large body of work, we know that moonlight improves visibility for avian predators, and that small mammals have a stable and predictable preference for refuge habitat in response to the indirect cue of predation risk (Abramsky et al. 2002, Orrock and Danielson 2004, Verdolin 2006). Seed-feeding carabids also preferentially use vegetated habitat (Shearin et al. 2008, Diehl et al. 2012), and periodic pulses in refuge use by small mammals likely induce behavioral responses at lower trophic levels. We also know that mice commonly feed on seeds as well as carabids (Whitaker 1966, Parmentor and MacMahon 1988, Birthisel et al. 2014) but the net impact of IGP on seed predation has never been tested experimentally (but see Davis and Raghu 2010).

In this experiment, we manipulated predation risk (i.e. moonlight), refuge availability (i.e. vegetative cover), and the presence of intraguild predators (i.e. small mammals) to isolate and quantify IGP and NCEs as they cascade from top predators (i.e. owls) to the seedbank. Invertebrates, rather than small mammals, are responsible for a majority of seed removal in similar systems (Menalled et al. 2000, Westerman et al. 2008, Ward et al. 2011). Thus we predicted that IGP by rodents would reduce top-down pressure on seeds, and that this effect would be mediated by rodents' use of refuge habitat under risky conditions (bright moonlight). We followed this field experiment with foraging assays in enclosed arenas where we evaluated carabid behavioral responses to both direct and indirect cues to predation risk by small mammals. This research illuminates complex, multi-directional effects of IGP and NCEs among taxa that fill common functional roles in ecosystems, advancing our knowledge about how non-linear trophic interactions shape community ecology.

4.3   METHODS

4.3.1   Field experiment

We performed this experiment in a randomized split plot design during the summers of 2013 and 2014 at Purdue University Meigs Research Farm near Lafayette, Indiana, USA. The main plot factor was moonlight, which we manipulated using 11 watt camping lanterns elevated to a height of 2m (similar to Abramsky and Rosenzweig 2002). Using a light meter, we verified that illumination on the soil surface in our plots was 1-2 lux/m, within ranges reported on clear nights with a full moon (Falkenburg and Clark 1998). Main plots were 18x18m with the lantern placed in the center of the plot, separated by at least 50m, and surrounded by soybean fields on three sides, with a grassy margin on the

fourth side. Fencing surrounded the entire experimental plot matrix, excluding large

mammals (i.e. deer) from the system.

Within each main plot, four 6x6m subplots were equidistant from the light source,

with 4.5m spacing between them. Subplot treatments included the fully-crossed

combinations of the remaining factors: presence/absence of cover and presence/absence

of vertebrates. Cover treatments were drill-seeded with red clover (*Trifolium pretense* L.)

in March 2013 at a depth of 1cm with rhizobium-inoculated seed at a rate of 13.5kg/ha.

We used red clover for our cover treatment because it is simple to manage as a

homogenous stand and common in landscapes where our focal taxa co-occur. The stand

of clover planted in 2013 was maintained for both seasons of this experiment, and

periodic mowing controlled seed rain. Bare soil treatments and margins received pre-

emergent herbicides (glyphosate, simazine, and oryzalin) in May and August of each year

to maintain a homogenous exposed environment without soil disturbance. We avoided

herbicide applications during pitfall sampling, but these herbicides have no reported toxic

or repellent effects on adult carabids (Brust 1990). Vertebrate exclusion plots were

fenced with 1cm hardware cloth, buried at a depth of 30cm, and topped with 20cm

aluminum flashing (per Bricker et al. 2010). Hardware cloth fencing is unlikely to inhibit

movement of invertebrates between plots (Parmenter and MacMahon 1988). To maintain

vertebrate-free environments, we live-trapped small mammals inside fenced plots for two

nights prior to sampling periods and moved any captured individuals >500m away from

the study site.

We measured the effects of all three experimental factors (moonlight, refuge, small

mammal exclosure) on dark nights (with <25% of the moon illuminated) over 7 days

each month, and then measured the effects of refuge and small mammal exclosure only (no moonlight factor) over the week of the full moon (>75% illumination), two weeks later. Experiments were performed over two complete lunar cycles in 2013 and three complete lunar cycles in 2014. See appendix for specific sampling dates.

4.3.2   Data collection

We quantified the activity density (a hybrid index of foraging activity and density) of carabid seed predators using two pitfall traps in the NW and SE sides of each plot, 2m from corners. Traps were open for 2-5 nights during each dark and light phase of the moon, depending on weather. Because carabid foraging activity is strongly influenced by temperature (Saska et al. 2013) and heavy precipitation floods pitfall traps in bare soil, traps were closed on nights with rain or temperatures below 10°C. Traps were dry with no killing agent, and live carabids were identified to genus, which enabled us to determine trophic guild. Captured beetles were added to a temporary lab colony for use in behavior assays.

To determine the effects of the experimental factors on seed predation we used seed removal assays of *Chenopodium album*, a common plant throughout temperate ecosystems, and palatable to both vertebrate and invertebrate granivores (Lundgren and Rosentrater 2007). Assays were performed in each plot using inverted petri dishes covered with double-sided carpet tape (as in Ward et al. 2011), with 100 *C. album* seeds evenly distributed, then covered with sand, and placed flush with the soil surface. Petri dishes were covered with a plastic rain guard to protect them from weather and discourage avian seed removal, and remained in the field for 7 nights during dark ($< 25\%$ illumination) and light ($> 75\%$ illumination) phases of the moon. Seed removal assays

continued for an additional moon cycle in 2014 after pitfall sampling terminated. Seeds remaining after 7 nights were counted with a dissecting microscope to give proportion of seeds removed for each plot. To assess seed loss due to abiotic factors and handling error, we used control dishes that were covered by fine mesh; however, seed loss was always minimal ($< 5\%$). Although seed removal does not always lead to seed predation (VanderWall et al. 2005), we assumed that animal-mediated secondary dispersal is relatively unimportant for *C. album*, due to its high palatability and physical evidence of consumption on-site.

4.3.3   Carabid behavior assays

To assess NCEs of rodents on carabid seed consumption and foraging activity, we performed behavior assays in enclosed arenas using *Harpalus pensylvanicus* DeGeer, the most common beetle at the research site. We assembled foraging arenas in 0.5 m^2 plastic totes with lids, each of which was lined with sand and contained a water source. We simulated ambient seed rain density of *C. album* by distributing seeds (2.5 g dry weight) evenly on the surface, and four adult *H. pensylvanicus* individuals were starved for 48 hours and added. Carabid densities were similar to those used by Prasad and Snyder (2004) in another foraging study.

Beetles were subjected to three different predator treatment combinations: 1) A 'caged predator' treatment had a live *Peromyscus* individual (collected the previous evening at the field study site) in a mesh enclosure with food and water near the edge of the foraging arena, assessing NCEs induced by direct cues of predation risk. 2) An indirect, 'olfactory cue' treatment included an empty sherman trap that was used to capture mice the previous evening, and 3) a 'predator free control' quantified foraging

behavior in the absence of predation risk. The predator-free treatments contained a sham cage so that the same surface area would be covered in all treatments. We also included two refuge treatments: bare and red clover clippings, which quantified effects of structural refuge on beetle activity and seed predation. In the refuge treatments, red clover clippings were placed in three thin patches 15cm in diameter. While they provided some structural cover, beetles were always visible beneath.

The fully-crossed behavior experiment resulted in 6 different treatment combinations. We conducted one fully-crossed replicate per night, and repeated the experiment on 5 nights using different beetles and mice. At dusk, seeds, beetles and mice were added to the arenas in their respective combinations, and the experiment lasted 12 hours. Seed consumption by beetles was measured by sieving and taking dry weights of seeds remaining at the end of the assay. Beetle activity was measured using night-time surveillance cameras mounted over holes in the lids of the foraging areas, and a DVR system (Q-See QT228-8B5-5). After a three hour acclimatization period, 5 minutes of footage was viewed and analyzed every other hour beginning at 23:00 and ending at 05:00, yielding 20 minutes total footage per replicate. Movement was quantified by pausing the video and recording time at each moment where one of the beetles stopped or started moving. We summed the amount of time per video assay that each of the four beetles spent moving and converted that to a proportion of time spent active.

4.3.4   Statistical analysis

We examined the effects of our treatments in the field experiment using an experimental moonlight model with data collected only on dark nights (<25% illumination), and a separate model comparing refuge and IGP effects under bright,

ambient moonlight (>75% moon illumination). In the experimental moonlight model, main effects and interactions between the three factors (cover, light, vertebrate exclusion) on invertebrate activity density were evaluated with a mixed–effect generalized linear model (GLM) assuming a poisson distribution using the lmer function in the lme4 package of R (R Development Core Team 2013). Pitfall trap sums of individuals from all seed-feeding carabid genera (according to Lundgren 2009) were the response variable, and fixed effects were cover, experimental moonlight (lanterns), vertebrate exclusion, and number of nights traps were open. Random effects were sample date nested in year and subplot nested in main plot. A similar analysis was performed for pitfall trap data collected under bright ambient moonlight, except that the 'light' variable was removed from the model (as all treatments were exposed to bright conditions).

Seed removal assays were analyzed with a similar approach to carabid activity density, using separate models for dark nights (including experimental moonlight as a factor) and moonlit nights (where only refuge and exclosure were evaluated). We used mixed-effect GLMs assuming a binomial distribution, with proportion of seeds removed as the response variable. Cover, experimental moonlight, and vertebrate exclusion were fixed effects, and random effects were sample date nested in year and subplot nested in main plot.

For the foraging behavior assays, we analyzed mass of seeds eaten and movement using MANOVA, with predator treatment and refuge treatment as predictor variables, blocked by replicate. Proportion of time spent moving was arcsin-square root transformed to meet the assumptions of the model.

4.4   RESULTS

4.4.1   Carabid activity density

Over the two sampling seasons we collected 1,971 seed-feeding carabids, and more than 45% came from the genus *Harpalus*. *Poecilus* spp*., Anisodactylus* spp*., Pterostichus* spp*., Stenolophus* spp*.,* and *Cratacanthus* spp*.* comprised another 51% of the community. In the experimental moonlight model (on dark nights), we found a three way interaction between experimental moonlight, cover, and vertebrate exclusion across both years of the study (Fig 4.1, Table 4.1a), indicating that vertebrate predator pressure on carabid activity density was mediated by artificial moonlight and refuge availability. Activity density of carabid seed predators was up to 50% lower in red clover plots exposed to artificial moonlight when vertebrate predators were present (Fig 4.1a). In unlit bare plots, vertebrate predators caused a 58% reduction in carabid activity (Fig 4.1a). In the ambient moonlight model, we found a cover*vertebrate exclusion interaction, consistent with effects observed using artificial moonlight (Table 4.1b), with a 35% reduction in activity density in refuge plots due to vertebrate predators (Fig 4.1b).

4.4.2   Weed seed predation

In the experimental moonlight model, cover was the only factor that affected weed seed predation (Coefficient= 2.36, z=6.19, p= <0.001). Seed removal was approximately 42% higher in plots with red clover (Fig 4.2a), but there were no significant effects of vertebrate intraguild predators, experimental moonlight nor any interactions of these variables. On nights with bright ambient moonlight, cover also affected seed removal (Coefficient=2.35, z=7.01, p=<0.001), and all other variables had no detectable impact on net seed removal, although there was a non-significant 11%

reduction in seed removal in refuge plots exposed to vertebrate predators that was consistent across both years of the study (Fig 4.2b).

4.4.3 Foraging behavior assays

Predator treatment and refuge both impacted *Harpalus* activity and seed consumption, but did not interact (Table 4.2). Compared with the predator-free treatment, both caged mice and olfactory cues reduced movement by 50% (Fig 3a), but increased seed consumption by 45% (Fig 4.3b). Refuge availability decreased movement by 50%, and increased seed consumption by 40%.

4.5 DISCUSSION

Our experiment showed that IGP pressure mediated by refuge use weakened carabid activity density by 50% in both artificial (Fig 4.1a) and ambient (Fig 4.1b) moonlight. This reduction in activity due to vertebrate predators is similar in magnitude to those observed by Parmentor and MacMahon (1988), who examined carabid activity in response to vertebrate exclusion in a grassland system, but our experimental framework enables us to demonstrate that this response is mediated by cascading NCEs of top predators (i.e. owls). These patterns are likely to pervade many systems as seed-feeding insects are ubiquitous in both managed and natural landscapes (Nimela and Kotze 2009), and small mammals' response to the cue of moonlight is stable across diverse ecosystems (Orrock and Fletcher 2014).

Structural refuge has been shown to reduce pressure on intraguild prey by reducing predator/prey encounter rates (Finke and Denno 2002, Janssen et al. 2007, Schmidt and Rypstra 2010). It may also yield neutral effects (Grabowski 2004, Frago and Godfray 2014) or even facilitate IGP (DeVore and Maerz 2014), as our results suggest.

Many studies have documented carabids' preference for the structure, microclimate, and superior food resources of vegetated habitats (Carmona and Landis 1999, Gallandt et al. 2005, Diehl et al. 2012), but these amenities likely come at a cost of intensified IGP risk under conditions that drive increased use of shared refuge habitat by their vertebrate predators.

Our foraging behavior assay of *H. pensylvanicus* revealed that the observed reductions in carabid trap capture due to IGP can be partially explained by suppressed activity (Fig 4.3b), which we measured using olfactory cues as well as direct cues from caged mice. Emigration from plots where beetles were vulnerable to vertebrate predators could also explain predator-mediated reductions in trap capture (Moran and Hurd 1994, Walzer and Schausberger 2013).

We expected that the strong IGP-mediated reductions in carabid activity density (Fig 4.1) would dampen trophic cascades (sensu Finke and Denno 2004), relaxing top down pressure on seeds, but found little evidence of this in the field (Fig 4.2). Still, even when both vertebrate and invertebrate seed predators had access to weed seeds in open plots, removal rates were no higher than those where vertebrates were excluded, consistent with other evidence of predator interference (Snyder and Wise 1999). Less than additive effects of the two seed predator groups suggest that IGP indeed reduces top-down pressure on weed seedbanks, although this was not directly affected by refuge use by small mammals.

NCEs generally strengthen trophic cascades (Preisser et al. 2005) because prey often respond to risk with reduced foraging (Schmitz et al. 2004). In strong contrast to our predictions, carabids actually consumed 45% more seeds in the presence of direct and

indirect predator cues (Fig 4.3a), even though they mounted a clear predator avoidance strategy (suppressed movement; Fig 4.3b). Compensatory feeding in response to predation risk has been documented in other insects; Thaler et al. (2012) showed that after a period of reduced feeding, *Manduca sexta* compensated later with increased foraging despite the continual threat of predation. Grasshoppers also demonstrate compensatory feeding on food sources with a higher C:N ratio in response to predation risk due to increased vigilance, metabolism, and carbohydrate requirements (Hawlena and Schmitz 2010). For the most part, carabids are omnivorous (Lundgren 2009) and capable of shifting their diets in response to varying risk and resource availability. Seeds are reliably available within relatively large patches (Cardina et al. 1997), and may be an excellent choice of 'stress food' for carabids compared to other resources that require more active foraging, such as insect prey.

The weak effects of NCEs on seed consumption we observed in our field study could be explained by the compensatory feeding documented in the foraging arena; this could potentially overcome any relaxed top-down pressure due to IGP in our system. The scale of our arena study was not large enough to produce a meaningful estimate of consumptive effects (due to direct IGP events) on carabid activity and seed consumption, but a simulation model estimated a 17% reduction in seed consumption by carabids due to predation by vertebrates (Birthisel et al. 2014). Consumptive effects of IGP combined with contrasting effects of compensatory seed feeding by carabids as well as granivory by vertebrates themselves may yield a net neutral impact on seed removal, as we observed in the field, despite clear behavioral responses to treatments by both invertebrate and vertebrate taxa.

Although moonlight is a well-known predictor of predation risk (Verdolin 2006), no existing work examines complex downstream effects of predator avoidance over greater than three trophic levels. Most studies that examine NCEs use highly simplified food webs over a short timescale, and omnivory can disrupt NCEs cascades, just as it does in conventional food webs. When predators and prey share a food source, cascading NCEs can yield a negative or neutral effect on net consumption, as we observed in our study. We found that avoidance of top predators via refuge use induced avoidance of intermediate predators by carabids, which produced perplexing, contrasting effects on top-down pressure. Moonlight provides optimal hunting conditions for top predators, which influence foraging strategies of intermediate predators, who balance their own risk/foraging tradeoffs while maintaining a dynamic response to prey (Pentariani et al. 2014). Prey also make dynamic foraging decisions, accounting for varying risk from multiple sources (Orrock and Fletcher 2014). In a world where 'trophic promiscuity' is the norm (Hunter 2009), it is important to follow suit and rigorously evaluate the multi-directional impacts of animals that operate from the perspective of both predator and prey (Lima 2002).

Acknowledgements

4.6   REFERENCES

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Table 4.1 Results of mixed-effect GLMs of granivorous carabid pitfall trap captures a) dark nights and b) moonlit nights

Factor	Coefficient	SE	Z	P	
d) Granivore capture on dark nights					
Intercept	0.27973	0.4273	0.655	0.512	
Trap nights	0.20853	0.0642	3.25	0.001	**
Cover	0.57434	0.1922	2.989	0.002	**
Vertebrates	-0.2985	0.2198	-1.358	0.174	
Lantern	0.38808	0.1954	1.986	0.046	*
Cover * vertebrates	-0.30456	0.2928	-1.04	0.298	
Cover * lantern	-0.17446	0.2485	-0.702	0.482	
Vertebrates * lantern	-0.55047	0.2970	-1.854	0.063	.
Cover * vertebrates * lantern	1.1727	0.3837	3.056	0.002	**
e) Granivore capture on bright (>75% moon illuminated) nights					
Intercept	0.04365	0.2952	0.148	0.882	
Trap nights	0.2085	0.0365	5.715	<0.0001	***
Cover	1.13027	0.2543	4.444	<0.0001	***
Vertebrates	0.19809	0.2728	0.726	0.467	
Cover * vertebrates	-0.76911	0.3613	-2.129	0.033	*

Table 4.2 Results of MANOVA testing effects of predator and refuge treatments on seed consumption and movement from behavior assays in a foraging arena.

Factor	df	F	P	
a) Seed consumption (g)				
Predator treatment	2	12.1498	0.000312	***
Refuge	1	23.9087	<0.001	***
Replicate	4	1.2155	0.333955	
Residuals	21			
b) Proportion of time spent moving				
Predator treatment	2	10.0157	<0.001	***
Refuge	1	5.1917	0.033262	*
Replicate	4	8.8622	<0.001	***
Residuals	21			
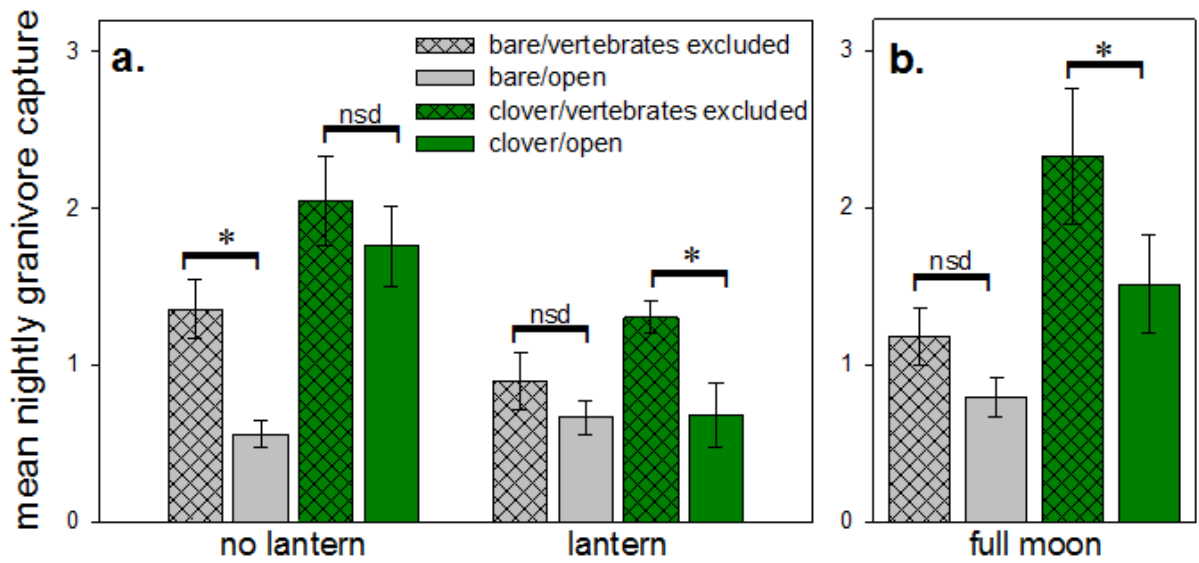
Figure 4.1 Mean (+/- SE) nightly pitfall capture of granivorous carabids pooled across years on **a)** dark nights with experimental moonlight treatments and **b)** full ambient moonlight. Significance of the effects of vertebrate intraguild predators at each light and cover level was determined using planned contrasts.
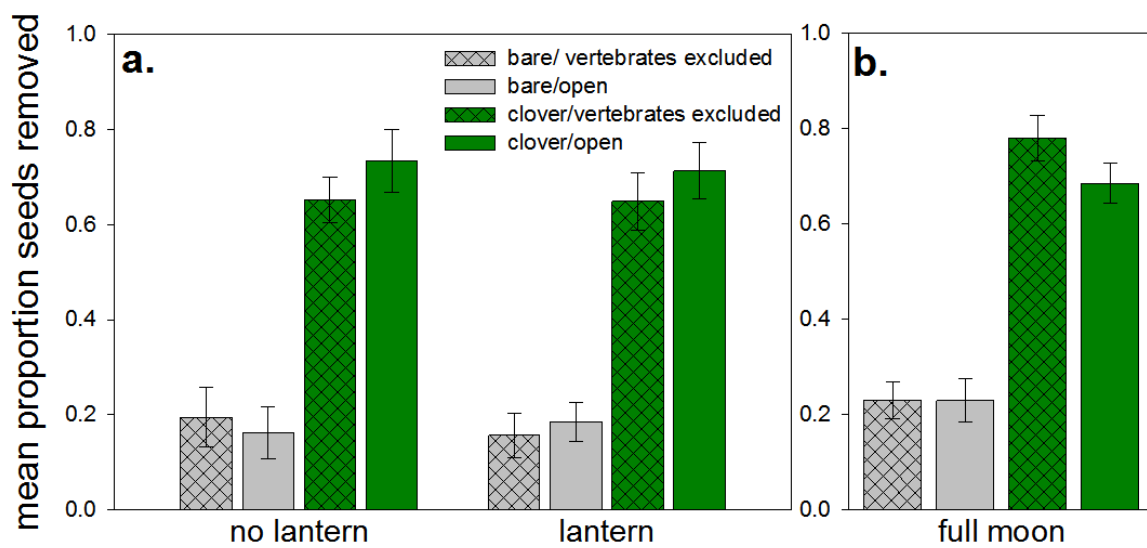
Figure 4.2 Mean (+/- SE) proportions of *Chenopodium album* seeds removed by seed predators over 7 day assays on a) dark nights with experimental moonlight treatments and b) full ambient moonlight.
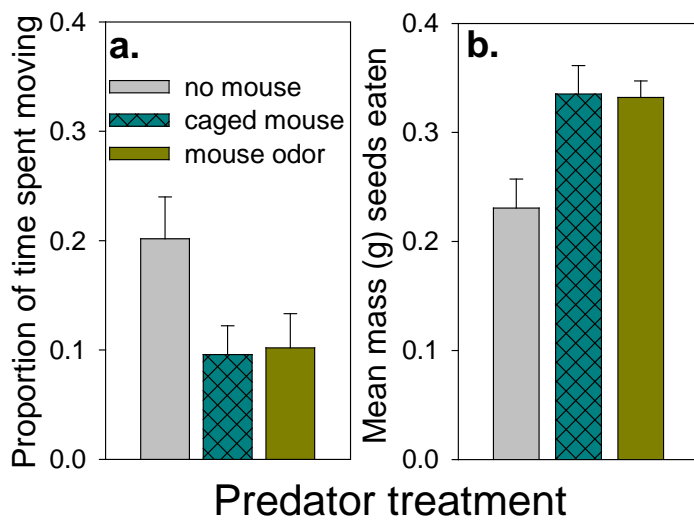
Figure 4.3 Mean (+/- SE) of a) mass of seeds consumed and b) proportion of time spent moving by four *H. pensylvanicus* individuals during a 12 hour foraging assay under three predation risk treatments.

Figure 4.4 Concept map of seed-based food web. Direct, consumptive effects are represented by black arrows; dashed grey arrows indicate predator-mediated indirect effects; thin grey arrows indicate potential NCEs.

Figure 4.5 Photo of the field site

CHAPTER 5.   SEED PREDATORS REDUCE WEED EMERGENCE UNDER
HIGH PROPAGULE PRESSURE

Running title: Seed Predators Reduce Weed Growth
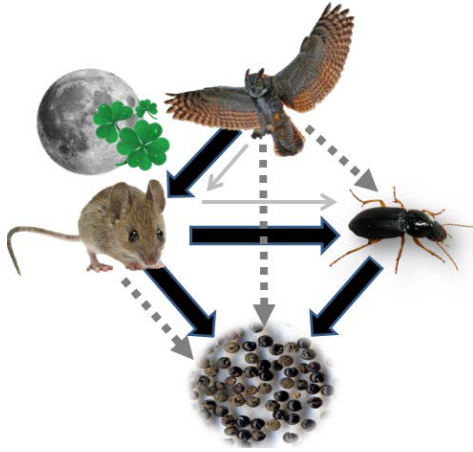
5.1   ABSTRACT

Weeds are selected for overwhelming propagule pressure, and while vertebrate and invertebrate seed predators destroy a large percentage of seeds, their ecosystem services may not be sufficient to overcome germination site limitations. Cover crops are suggested to facilitate seed predation, but it is difficult to disentangle reductions in weed recruitment attributable to granivores from those due to plant competition. Using common lambsquarters (*Chenopodium album*) as a focal weed species, we used experimental seed subsidies and differential seed-predator exclusion to evaluate the utility of vertebrate and invertebrate seed predators in fallow, killed cover crop, and living mulch systems. Over two growing seasons, we found that seed predators were responsible for a 38% reduction in seedling emergence and 81% reduction in weed biomass in fallow plots following simulated seed rain, suggesting that granivory indeed overcomes site limitation and suppresses weeds. However, the *C. album* densities in ambient seedbanks across fallow and cover crop treatments were high, and seed predators did not impact their abundance. Across the study, we found either neutral or negative effects of vertebrate seed predators on seed predation, suggesting that invertebrate seed predators contribute most to *C. album* regulation in our system. These results imply that

weed seed biocontrol by invertebrates can reduce propagule pressure initially following senescence, but other tools must be leveraged for long-term seedbank management.

Nomenclature: Common lambsquarters, *Chenopodium album* L. CHEAL

Keywords: weed seed predation, cover crops, *Chenopodium album*, seed limitation

5.2   INTRODUCTION

Crickets, ground beetles and small rodents are ubiquitous residents of crop environments, and all contribute to weed seed biological control. Their capacity to limit weed recruitment is frequently examined, particularly in organic systems where weed management is labor-intensive (Landis et al. 2005). Seed predators destroy a large percentage of weed seeds each year, but weeds are selected to produce overwhelming propagule pressure. For example, a single annual weed can approach 176,000 seeds per plant (Clements et al. 1996), and thus weed seed biological control can only have an agriculturally relevant impact if seed predation rates are sufficient to overcome germination site limitations (Lundgren 2009). Studies have shown that invertebrate seed predation can affect plant population dynamics in natural systems (Crawley 1992, Turnbull et al. 2000). Yet, seed limitations have not been clearly established in agroecosystems (Boyd and Van Acker 2004).

Numerous studies document high seed predation rates using episodic point estimates of seed removal (Gallandt et al. 2005, O'Rourke, et al. 2008, Fox et al. 2013), and simulation models suggest that seed predation is an important factor limiting seedbank flux (Westerman et al. 2006), but few connect the action of seed predators with subsequent weed growth (Brust 1994, White et al. 2007). Due to seasonal tillage requirements in most working agricultural systems, weed emergence rates are rarely

measured in growing seasons following seed predation assays, limiting our ability to precisely evaluate how seed predator activity ultimately drives weed demography (but see White et al. 2007). Nonetheless, the current body of literature has helped to identify local habitat factors that affect seed predator foraging. These include insecticide use, tillage regimes, proximity to untilled margins, and vegetative cover (Landis et al. 2000).

Both vertebrate and invertebrate seed predators utilize available vegetative resources like cover crops, and cover may facilitate weed seed destruction (Gallandt et al. 2005, Pullaro et al. 2006, Meiss et al. 2010). However, it is difficult to disentangle reductions in weed recruitment attributable to the indirect effect of habitat provisioning for granivores from the direct effect due to competition with cover crops. Living mulches consistently harbor higher activity densities of weed seed predators than exposed habitats (Carmona and Landis 1999, Davis and Liebman 2003, Shearin et al. 2008), but killed cover crop mulches have mixed effects on seed predator recruitment (Pullaro et al. 2006, Ward et al. 2011, Blubaugh and Kaplan 2015). Structural habitat complexity may provide shelter and improved microclimate for seed predators, but alternative food resources associated with living plant biomass are also important (Diehl et al. 2012). Despite evidence for stronger pressure by seed predators in vegetated habitats, suitable microsite reduction caused by cover may neutralize their effect on seedling emergence (Reader 1993).

Correlative evidence from the current body of literature suggests that seed predators perform important ecosystem services, and may even regulate weed seedbanks (Bohan et al. 2011). However, without experimental validation of their impact, growers may be reluctant to adapt management programs to promote stable seed predator populations. To that end, we performed an experiment to explicitly examine the effects of seed predation

on subsequent weed germination rates in common cover crop environments. We used seed additions of common lambsquarters (*Chenopodium album* L.) and differential exclosure treatments to compare the effects of vertebrate and invertebrate seed predators on emergence, biomass, and seedbank density in undisturbed plots over multiple field seasons. This work clarifies the role of seed predators in weed population dynamics, documenting weed seed biological control in a comprehensive, agriculturally relevant framework.

5.3 METHODS

5.3.1 Study System

The experiment was conducted at the Purdue University Meigs Horticulture Research Farm near Lafayette, Indiana, USA. We examined the effects of cover crop type (3 treatments), seed predator exclosure (3 treatments), and weed seed additions (2 treatments), in a randomized block, split plot design. The main plot factor was cover crop type (rye/vetch, red clover, and fallow), and these were planted in 8 replicated 9x12m plots (24 plots total) following disc tillage in early September 2012. Rye/vetch mix (*Secale cereale* L., and *Vicia villosa* R., 2:1 by weight) and red clover (*Trifolium pratense* L.) were planted at rates of 60 kg/ha and 13 kg/ha, respectively. Plots had 4.5m margins between them, which were planted to a perennial grass mix. The experimental plot matrix was surrounded on one side by a grassy margin and on three sides by conventionally managed soybeans. In May 2013, rye/vetch cover crops were killed at flowering when the stand was at ca. 246 g dry biomass/m by flail mowing and left as mulch on the soil surface. Weeds in the system were managed with monthly mowing, and no chemical inputs were used throughout the study.

Four weeks after cover crops were sown, six circular, 0.5m^2 subplots were established 2m apart along a transect running lengthwise across the north side of main plots, 2 m from the border (Fig. 5.4). Subplot treatments were the crossed combinations of seed subsidy treatments (presence/absence), and differential exclosure treatments (vertebrate exclosure, complete exclosure, and open control), randomized within each main plot. Small mammals were excluded using 1m tall hardware cloth fences (1 cm gauge); both small mammals and vertebrates were excluded using 80 cm aluminum flashing. Both fence types were buried 5 cm deep. Birds were not excluded from subplots because we assumed their contribution to weed seed predation would be minimal (Holmes and Froud-Williams 2005). To evaluate the seed limitation in our system, we evenly sowed 10 grams of *C. album* seeds (about 15,000 seeds total) in half the plots on 19 October 2012 and 27 September 2013. Seed additions were intended to approximate the timing and density of biologically relevant, yet intense *C. album* seed rain in the fall, and were purchased from Azlin Seed Service (Leland, Mississippi, USA). We chose *C. album* as a focal species because it was the most common weed at our research site, and also because of its global importance as a persistent weed in temperate agricultural systems (Holm et al. 1977).

Due to poor clover germination in fall 2012, installation of all clover subplot treatments were delayed until September 2013, when the red clover stand was ca. 288 g dry biomass/m. To account for this methodological difference, we established a second complete replication of subplot treatments in the fallow plots in 2013 to coincide with the delayed clover treatments for comparison. These were placed on the south side of fallow plots, which were tilled two weeks prior. Thus, fallow plots were compared with killed

rye/vetch treatments in 2012, and a second fallow replication paired with clover

treatments in 2013. Eighteen of the twenty-four cover and fallow plots were used in the

second year of the study.

5.3.2   Data collection

The variables we measured were spring weed emergence (given as stem counts

per subplot), harvested weed biomass, and seedbank density of *C. album*. We counted

seedlings on 23 May 2013 and 8 June 2014, and harvested biomass on 26 September

2013 and 8 June 2014. Biomass was dried for 72 hours then weighed. We had to remove

two plots from our biomass analysis where the open control treatments were accidentally

mowed. To avoid edge effects in subplots, seedlings within 10 cm of the fence

boundaries were not counted nor harvested. After data were collected, all *C. album* plants

were removed from subplots and surrounding areas to prevent seed rain.

Seedbank samples from all plots (those established in both years of the study)

were collected on October 1 and 2, 2014. Five soil samples were taken in each subplot

using a soil core (5cm depth, 10 cm diameter). No soil was collected within 10 cm of

each subplot boundary to avoid edge effects. *C. album* seeds were washed from a 625 mL

subsample of the 5 homogenized cores using a 0.595 mm sieve. Prior to counting, we

crushed dried seed samples against the sieve, eliminating decayed seeds. Unbroken seeds

were separated from debris, identified and counted using a dissecting microscope.

5.3.3   Statistical analysis

C. album seedling counts from fallow plots were analyzed using a mixed effect

generalized linear model assuming a poisson distribution with quasi-likelihood estimation

(for overdispersion) using the glmmPQL function in the MASS package of R (R

Development Core Team 2014). Red clover and rye/vetch plots were excluded from

seedling and biomass models because we only observed quantifiable *C. album* growth in

fallow plots. Fixed effects were exclosure type (open, invertebrates only, mammals +

invertebrates excluded), seed subsidy, and year; main plot was a random effect. *C. album*

biomass values from fallow plots were natural log transformed and analyzed with a

mixed effect model. Fixed and random effects were the same as they were for seedling

counts, and the lme function in the nlme package of R was used. Seedbanks were

analyzed using a separate model for each year of the study, since cover crop treatments

could not be fully crossed within years due to poor clover germination in year one of the

study. *C. album* seedbank densities were natural log transformed and analyzed with

mixed effect models, using the lme function in R. Fixed effects were cover crop type

(fallow and rye vetch in 2012, fallow and red clover in 2013), seed subsidy, and

exclosure type; random effects were main plot nested in block. Raw data from each plot

were analyzed for each response variable, but they were converted to normal metric units

(seedlings/m^2, seeds/mL, etc) for presentation in figures.

5.4   RESULTS AND DISCUSSION

Over repeated seasons, we found that seed predators overcame intense propagule

pressure and substantially reduced germination rates after fall tillage in fallow plots

(Table 5.1a), demonstrating that granivores perform relevant services that improve weed

control. In plots with seed subsidies, *C. album* emergence rates were almost twice as

when seed predators were excluded as they were when insects or rodents had access (Fig

5.1). The impact of seed predators on weed biomass was lower in plots with ambient

seedbanks; we found a seed*exclosure interaction on *C. album biomass* (Table 5.1b), but

the main effect of seed predator exclosure was significant overall for seedling emergence (Table 5.1a). The positive effect of seed addition on both germination and biomass provides evidence of seed limitation in annual cropping systems (Table 5.1a,b). The reductions in emergence due to weed seed predation that we observed were similar in magnitude to the only other study that explicitly measured it (White et al. 2007), even though the seed rain density we examined was higher by more than an order of magnitude, further validating the capacity of seed predators to overcome intense propagule pressure.

We intended to compare the effects of seed predators on *C. album* germination rates between cover crop types, but found too few seedlings across all cover crop treatments for analysis, even after removing mulch from the soil surface (data not presented). This suggests that competition with cover crops outweighs seed predator effects on weed germination. However, we were able to examine effects of seed predation at the seedbank level across the different cover crop treatments, and surprisingly, cover crops had no effect on the seedbank in either year (Table 5.1 c,d). Cover has strong effects on episodic seed removal (Hegenstaller et al. 2006, Meiss et al. 2010), thus we expected that granivores would reduce seed density in the upper soil profile (0-5cm) of cover crop treatments. The ambient *C. album* seedbank we observed was extremely high compared to those observed in other systems (Davis et al. 2012), which may have overwhelmed the effects of our treatments. Invertebrate seed predators have a limited capacity to extract seeds from beneath the soil surface (White et al. 2007, Harrison and Gallandt 2012), and while they obviously reduce the number of number of seeds in the shallow germinable profile of *C. album* (2.5 cm, Weaver 1988), their impact

may not be detectable at greater depths when underlying seedbanks are particularly abundant.

While the effect of seed addition was always positive and significant (Table 5.1 c,d), we were surprised by the small magnitude of the effect on the *C. album* seedbank (Fig 5.3), which was only 9% across both years of the study. Our seed subsidy treatments approximated intense seed rain, and *C. album* has relatively strong seedbank persistence (Davis et al. 2008), but made only a subtle contribution to seed density in the soil profile. Again, effects of experimental seed subsidies could have been diluted by dense and patchy ambient densities.

We detected no effects of seed predators on the *C. album* seedbank in the 2013 plots (Table 5.1d), which had only been installed for one year at the time of soil sampling. In the 2012 plots, we found a seed*exclosure interaction, two years after treatments were applied (Table 5.1c). When seed subsidies were added, significantly fewer seeds were recovered in open plots compared with both types of exclosure (Fig 5.3b). In unsubsidized plots, however, more seeds were recovered in plots where only invertebrate seed predators had access than in either of the other two exclosure treatments (Fig 5.3b). Our analysis provides weak evidence of *C. album* seedbank regulation by granivores after two years of continuous experimental exclosure. This contradicts the correlative evidence of Bohan et al. (2011), who regressed seasonal ground beetle pitfall trap captures with weed seedbank reduction. We expect that seed predators could have a much stronger effects in environments with lower ambient seedbanks, or with weed species that are less persistent.

There was no difference in weed emergence between the two types of seed predator exclosures, suggesting that small mammals contribute little to weed suppression in this system, consistent with other work performed in US Midwestern field crop environments (Menalled et al. 2000, Westerman et al. 2008). Other studies provide conflicting evidence that small mammals perform the majority of weed seed predation services (Westerman et al. 2003, Baraibar et al. 2009), and vertebrates are the only granivorous taxa whose seed predation services carry on over winter (Williams et al. 2009). We expected to see stronger effects of vertebrate exclosure, given that our experiment quantified seed predation across entire seasons, and because seeds are an important component of small mammal diets (Whitaker 1966, Flick 2013). Future research should identify environmental variables that predict their reliance on weed seed resources. When seasonally available, small mammals preferentially forage on spilled grain and invertebrate prey resources (Whitaker 1966); alternative food resource availability could divert feeding on weed seeds. Also, small mammals often prey on invertebrate seed predators (Davis and Raghu 2010, Birthisel et al. 2014). Even if small mammals consume a substantial amount of weed seeds, their effect on seedbank flux could be neutralized by intraguild predation on crickets and ground beetles. Intraguild predator effects may be intensified in cover crop environments, as rodents and granivorous insects both use vegetative cover as refuge and overwintering habitat (Gallandt et al. 2005, Moorman et al. 2013). This could provide additional explanation for the weak effects of cover crops we observed (Table 5.1c,d). Nevertheless, cover crops are critical ecological tools for weed suppression, and their myriad benefits certainly outweigh any costs of intraguild predation among seed predators.

Our results validate the utility of beneficial seed-feeding insects by demonstrating a 38% reduction in weed germination specifically attributable to seed predators. Still, seeds are only accessible by invertebrate seed predators as long as they remain on the soil surface, and while their ecosystem services are critical following seed rain (Figs 5.1 and 5.2), they become negligible once seeds are incorporated in the soil (Fig 3, Westerman et al. 2006). Our work suggests that high underlying seedbanks may overwhelm the seasonal impacts of seed predators, thus other tools must be combined for long-term seedbank management (Liebman and Gallandt 1997, Davis 2006). Integrated management strategies that promote seed predator activity following senescence (Landis 2005) while simultaneously targeting underlying seedbanks (Gallandt 2006) will lead to stronger suppression over time, and cover crops are critical tools for the task. They target weeds both above and below the soil surface by preventing emergence, promoting seed decay (Mohler et al. 2012), and providing optimal foraging habitat for seed predators (Landis et al. 2005).

Acknowledgements

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Table 5.1 Output from decomposed mixed models of *C. album* seedling counts (a), biomass (b), and seedbank density (c and d).

| Factor	Coefficient	SE	t	P	
a) Seedling counts (fallow plots only)					
Intercept	899.932	401.275	2.242	0.028	*
seeds	0.548	0.186	2.934	0.004	**
fence.invert	-0.475	0.228	-2.077	0.041	*
fence.open	-0.503	0.228	-2.201	0.030	*
year	-0.446	0.199	-2.238	0.028	*
b) Biomass (fallow plots only)					
Intercept	209.542	82.309	2.545	0.014	*
seeds	0.131	0.064	2.022	0.049	*
fence.invert	0.003	0.064	0.057	0.954	
fence.open	0.011	0.066	0.1761	0.860	
year	-0.104	0.040	-2.544	0.014	*
seeds:fence.invert	-0.142	0.091	-1.548	0.128	
seeds:fence.open	-0.189	0.094	-2.012	0.050	*
c) Seedbank density 2012 plots (rye/fallow)					
Intercept	4.661	0.315	14.768	<0.001	**
cover	0.463	0.334	1.388	0.207	
seeds	0.434	0.206	2.103	0.038	*
fence.invert	0.382	0.219	1.747	0.084	.
fence.open	0.356	0.219	1.625	0.108	
seeds:fence.invert	-0.668	0.253	-2.642	0.010	*
seeds:fence.open	-0.499	0.253	-1.972	0.052	.
d) Seedbank density 2013 plots (clover/fallow)					
Intercept	4.402	0.273	16.081	<0.001	**
cover	0.184	0.367	0.501	0.637	
seeds	0.273	0.100	2.725	0.008	**
fence.invert	-0.005	0.122	-0.043	0.965	
fence.open	-0.049	0.122	-0.406	0.685	
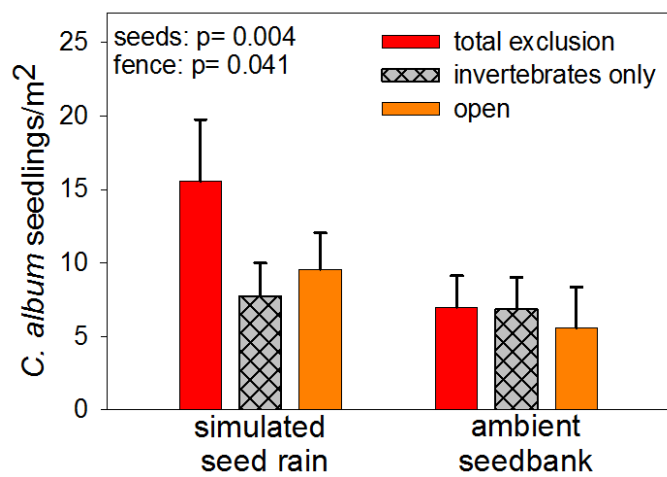
Figure 5.1 *Chenopodium album* seedling counts per m^2 in fallow subplots pooled from spring 2013 and 2014
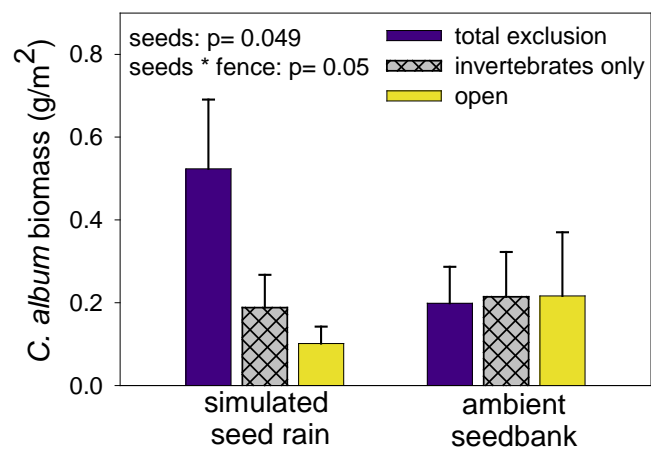
Figure 5.2 *Chenopodium album* biomass per m^2 in fallow subplots from spring 2013 and 2014
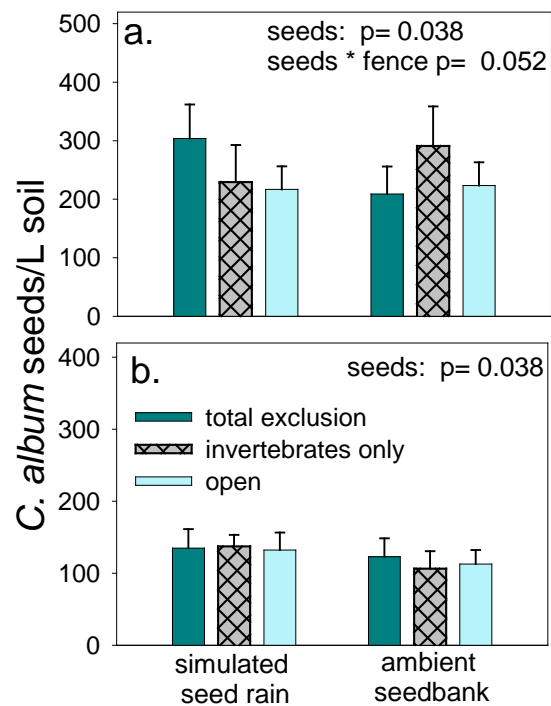
Figure 5.3 *Chenopodium album* seed density in soil samples a) pooled rye/vetch and fallow subplots established in 2012 and b) pooled clover and fallow subplots established in 2013. Seedbank samples were collected from all plots in September 2014.

Figure 5.4 Photo of field site when seed predator exclosures were installed in September 2012

Figure 5.5 Photos of field site in summer 2013: examples of a) Rye/vetch and b) fallow plots.
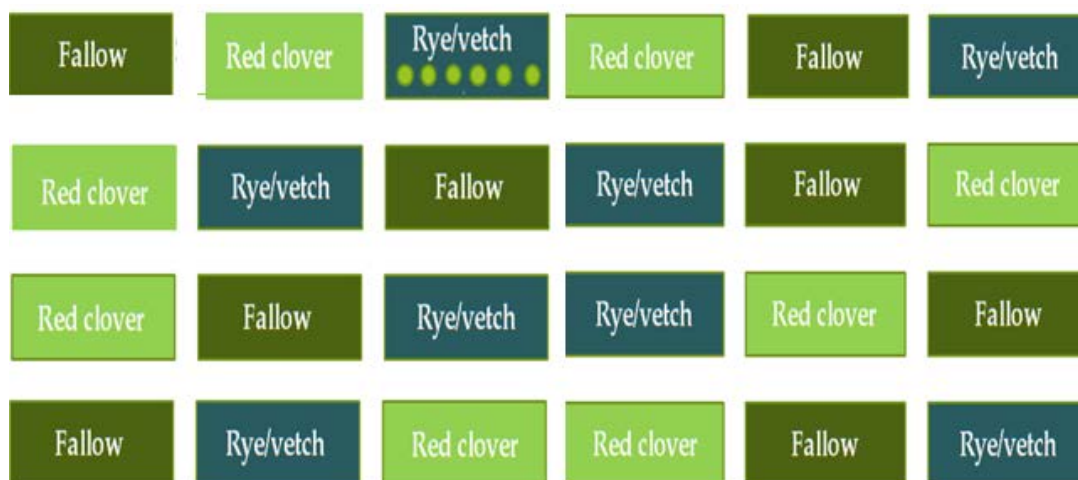
Figure 5.6 Diagram of the complete plot matrix

VITA

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Blubaugh, C.K. 2012. Meet the (beneficial) beetles: *Harpalus pensylvanicus,* weed seed predator. Purdue Vegetable Crops Hotline. 559:2.

INVITED PRESENTATIONS:

Tippecanoe Co. Library, Lafayette, IN (5/6/2015)
"Love your bugs: insectary plantings to attract predators, parasitoids and pollinators"

Tippecanoe Co. Master Gardener's Association, Lafayette, IN (5/6/2014)
"Love your bugs: insectary plantings to attract predators, parasitoids and pollinators"

Indiana Small Farm Conference, Danville, IN (2/20/2014)
Growing Organic workshop: Insect pest management ~ 50 farmer and extension educator participants

Rush Co. Master Gardener's Association, Rushville, IN (3/19/2012)
"Beneficial insects in the garden" ~30 Master Gardener participants

Boswell elementary school, Boswell, IN (4/5/2012)
"Bugs in the garden" 40 k-6 grade participants

PRESENTATIONS:

Entomology 2014: Portland, OR (11/2014)
"Does fear beget fear? Examining anti-predator cascades over four trophic levels"

MOSES Organic Farming Conference LaCrosse, WI (2/2014)
"Harnessing farm wildlife for weed management: Measuring suppression by rodents and insects" (poster)

Indiana Small Farm Conference Danville, IN (2/2014)
"Mechanical weed management tactics compromise recruitment of larval weed seed predators"

Indiana Small Farm Conference Danville, IN (2/2014)
"Harnessing farm wildlife for weed management: Measuring suppression by rodents and insects" (poster)

Entomology 2013: Austin, TX (11/2013)
"Fear-mediated cascades over four trophic levels affect seed predator function"

2nd International Symposium on the Molecular Detection of Trophic Interactions Lexington, KY (5/2013) "Predator-mediated habitat shifts affect weed seed predation"

Indiana Academy of Science: Annual meeting Indianapolis, IN (3/2013)
"Predator-mediated habitat shifts affect weed seed predation in an agroecosystem"

Indiana Small Farm Conference Danville, IN (3/2013)
"Seed predators reduce weed germination in cover crops" (poster)

Great Lakes Vegetable Working Group West Lafayette, IN (2/2013)
"Cover crops and baby carabids: how does larval environment shape beneficial insect communities?"

MOSES Organic Farming Conference LaCrosse, WI (2/2013)
"Seed predators reduce weed germination in cover crops" (poster)

Local Growers Guild Webinar (1/2013)
"A brief tour of new organic research at Purdue" (Viewed online by growers throughout IN)

Entomology 2012: Knoxville, TN (11/2012)
"Why do carabids love cover crops? Isolating mechanisms of recruitment in agroecosystems"

MOSES Organic Farming Conference La Crosse, Wisconsin (2/2012)
"Cooperating with carabids: in-field strategies for enhancing weed seed predators" (poster)

Entomology 2011: Reno, NV (11/2011)
"Carabid phenology, diversity and response to weed cover in a turfgrass ecosystem." (poster)

Plant Population Biology in a Changing World Bern, Switzerland (5/2009)
"Effects of Insect Herbivores on *Cirsium arvense* in the Native and Invasive Ranges" (poster)

SYMPOSIA ORGANIZED:

Entomological Society of America Plant-Insect Ecosystems section symposium (11/2014)
"Non-lethal effects of predators in arthropod food webs: ecological patterns, behavioral mechanisms and agricultural applications"

SERVICE:

Entomology Outreach Volunteer (2010-present) Purdue University, West Lafayette, IN
Master Gardener (2010-present) Tippecanoe Co. Master Gardeners Association, Lafayette, IN