AN ABSTRACT OF THE THESIS OF

Jessica M. Green for the degree of Master of Science in Horticulture presented on October 22, 2010.

Title: Structuring Habitat to Conserve Ground Beetles (Coleoptera: Carabidae) and Reduce Summer Annual Weeds in Agroecosystems

Abstract approved:

_________________________________________________________
R. Edward Peachey

Weed management in sustainable farming systems often requires the integration of several different pest management techniques. Cultural, biological, and chemical pest control efforts can be utilized to contribute to the common goal of reducing weeds in vegetable row crop systems. This research addresses how common disturbances such as tillage and insecticide use affect: carabid beetle assemblages; predation of weed seeds by invertebrates; and weed seed recruitment. Field experiments were conducted over three years at two different locations on the OSU Vegetable Research Farm, Linn Co., OR. We found that activity-density of carabid beetles varied seasonally and peaked in late August-September each year. Insecticide applied in year 1 affected seed loss in year 2, suggesting possible long-term effects of land management on weed seed removal. Weed recruitment was highly variable between treatment, site, and year. Conserving biological weed control agents in combination with cultural techniques such as reducing tillage and the use of cover crops, helps growers shift from expensive, density-independent control efforts to more ecological, long-term solutions for weed management in agroecosystems.
Structuring Habitat to Conserve Ground Beetles (Coleoptera: Carabidae) and Reduce Summer Annual Weeds in Agroecosystems

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Jessica M. Green, Author
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Dr. Ed Peachey provided his expertise in weed management to help develop the introduction section of Chapter 3. Also, Dr. Dan McGrath shared with me his extensive collection of literature regarding IPM in vegetable production systems.
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DEDICATION

This work is for my Father,

D.L.G.

1949-2008

“ That...was a great game. ”
Chapter 1 – Introduction

BIOLOGY AND DIVERSITY

Worldwide, there are over 30,000 described species of carabid beetles, making them one of the most abundant and diverse family of insects on Earth (Gullen and Cranston 1996). Because of this diversity, carabids make good subjects for taxonomic analyses (Maddison 1999) and ecological biodiversity research (Altieri 1999). Additionally, carabids have been studied as bio-indicators of overall ecosystem health (Pearsall 2007, Raino and Niemelä 2003) and because they are ubiquitous, provide a measurable response to how land disturbance influences existing invertebrate communities. Carabid beetles are present on every continent and found from sub-Arctic climates to deserts, to tropical and temperate rainforests (Theile 1977). Most Carabidae are nocturnal, but there are notable exceptions, as in the daytime-active Amara genus. Carabids are often called ground beetles because they spend most of their time on or below the soil surface. Flight muscles and wings are brachypterous (reduced) in most individuals but can be evolutionarily recovered as needed (Maddison 1999).

Carabids produce either one or two generations per year. Eggs are laid singly in the soil and females lay anywhere from 3-600 eggs in one season (Theile 1977). There are three larval stages for most species, and the last instar prepares for pupation by digging its own pupal chamber in moist soil (Lundgren 2009). Annual rhythms determine time and length of reproduction (Fadl 1996, Theile 1977). There are two main reproductive strategies in carabids; spring-breeders and autumn-breeders (Lindroth 1964). Spring-breeding taxa such
as Amara spp., and Harpalus affinis, emerge and mate in early in the spring, develop their larvae over the summer, and overwinter in the soil as adults. Conversely, Pterostichus melanarius, Anisodactylus binotatus, and Harpalus pensylvanicus exemplify beetles that mate in late-summer to autumn and spend the winters as larvae. Understanding life cycles is an important research concept, and subsequent sections will discuss how reproductive timing may affect abundance, survival, and dietary consumption.

Habitat choice differs between genera, and even species. Researchers from Hungary studied ground beetles based on habitat preference and found that opportunistic carabids such as Pterostichus melanarius, Anisodactylus spp. and Amara spp. were able to successfully thrive in urban environments while other specialist beetles were restricted to either forest or open-habitat areas (Magura et al. 2004). This concurs with Miguel Altieri’s theory that there is a degree of association between a species and the niche that they fill (1999). The relationship between a habitat and its colonizers is sometimes categorized by a preference index (Hatten 2007). Hatten found that large species like P. melanarius display a slight preference (V=0.2) for undisturbed areas, whereas Bradycellus congener (V=0.6) and other small species prefer looser soil. Amara spp. were listed as having a V-value equal to 0.0, indicating there were equal numbers of beetles caught in disturbed and undisturbed areas. Harpalus pensylvanicus, on the other hand, generally prefers more weedy areas (Forbes 1880, Menalled 2001, Tonhasca 1993).

Proximity to landscape features is another factor influencing ground beetle surveys (Dennis 1994). Studies conducted on the emigration of Pterostichus melanarius indicate that movement increases linearly with both temperature and level of starvation (Raworth 2001), and that colonization usually occurs from field edges inward (Thomas 1998, Nash 2008).
Some *Amara* species exhibit seasonal emigration in late summer by flying towards forest silhouettes (Fawki 2005). Dispersal power is high in ground beetles, and estimates of movement range from 29m/day (Raworth 2001) to 63m/day (Fadl 1996).

**SAMPLING AND STATISTICS**

Actual field density of ground beetles can vary greatly by region and cropping system, estimates range from 10-25 beetles/m² in agricultural fields. True carabid populations are often difficult, if not impossible, to estimate on a large scale. Some researchers use direct sampling techniques, which involve burying either wooden or metal arenas into the soil and collecting all beetles from within the arena (Clark 1995). This provides an estimate of absolute-density of the number of beetles per given unit area. Visual quadrant sampling is also a way to directly quantify carabid density. However, these techniques are highly labor-intensive and thus not practical for large-scale, long-term experiments. Therefore, it has become accepted practice to estimate population density via pitfall sampling (Spence and Niemelä 1994, Pedigo 1994, Brust and House 1988).

Pitfall traps are glass or nested plastic cups that are sunk into the soil so that the cup edge is level with the soil surface. There is often a killing agent such as soapy water or ethylene glycol within the cup. Insects fall into the pitfalls and are unable to escape. This type of passive sampling provides a relatively inexpensive and simple way to sample large areas of terrain simultaneously, and can provide valuable estimates of species richness and abundance of surface-dwelling arthropods (Carmona and Landis 1999). Continuous pitfall sampling has the distinct advantage of being able to reveal seasonal changes in activity peaks (Thomas 1998). There are, however, pitfalls to this methodology, pun intended. Abiotic aspects of the sampling area such as temperature and microclimate (Honek 1988, Clark
1995) can influence rate of capture. Areas with high amounts of vegetative cover also tend to underestimate carabid abundance (Greenslade 1964, Philips 2005). Additionally, biotic factors including beetle behavior (Booij 1994), aggregation tendencies (Pearce 2006, Thomas 2006), and prey availability (Chiverton 1984, Taylor 2008) can influence sample data. The term activity-density (AD) is used to reflect these differences and provide an estimate of movement and relative abundance of ground beetles.

Pitfall catches are often compared to other biotic observations in the field (Rainio and Niemelä 2003). Menalled et al. found a strong linear relationship ($r^2 = 0.70$) between the number of beetles per pitfall per day and the percentage of onion fly pupae removed from feeding trays (1999). A study from Illinois notes that predation of wax moth larvae was significantly correlated ($p \leq 0.01$) with quadrant sampling of arthropod predators but was not significant ($p=0.66$) when compared to pitfall catches (Lundgren 2006). Conversely, amending plots with grass seed seemed to enhance carabid AD as measured by pitfall traps ($p=0.003$), but was not significant ($p=0.096$) when compared to quadrants (Frank 2007). Decisions regarding experimental design and sampling method of a study should be based on the nature of the information to be gleaned.

Analyses of numerical count data present unique challenges and often require more sophisticated statistical measures (Sileshi 2006, White and Bennetts 1996). Ground beetles, in particular, are known to exhibit behavioral aggregation (French and Elliott 1999). Spatial clumping of individuals seriously violates the underlying assumptions of ANOVA, where sampling is intended to occur from a randomized, normal, equally-distributed population. Because of these violations, insect counts are often transformed by subjecting the raw data to log-normal, Poisson or negative binomial distribution (NBD). Pearce and Zalucki (2006)
found that while the Poisson and NBD were best for analyzing foliar-dwelling pest species, log-normal transformation (\( \ln(x+1) \)) was most accurate for describing the structure of four of the five studied groups of Coleoptera, including Carabidae. If there is obvious species dominance within a sample population, species are often analyzed separately (Gauch 1985), or assigned an over-dispersion parameter (\( \kappa \)) such that the variance (\( Y \)) changes from a linear relationship, where \( Y = \sigma^2 \), to a dependent measure, \( Y = \mu + \kappa \mu^2 \) (Sileshi 2006).

This reduces the amount of variability between common and rare species so that treatment effects can be tested without having to separate the data set.

**GROUND BEETLES IN AGROECOSYSTEMS**

Ground beetles found in farmland are one of the few groups of insects that are considered invasive yet beneficial. They were likely imported from Europe in the late 18th century in the soil in ballasts of trade ships (Theile 1977). More recent introductions to the Pacific Northwest may have occurred via potted nursery stock (Kavanaugh and LeBonte 2008). Although Carabidae is an incredibly diverse family overall, species diversity is not as high in agricultural settings it is in undisturbed areas. In fact, it is not unusual for only four to six species to comprise 75% or more of the total carabid community in arable land (Tonhasca 1993). Ground beetles that are present in agricultural land are usually opportunistic and adapt to the environment better than carabids found in stable habitats such as forests or prairies (Rainio and Niemelä 2003).

Many carabids are generalist predators and are reported to effectively reduce populations of agricultural pests (Symondson 2002), including slugs (Fawki 2005). In an extensive review of laboratory feeding experiments, carabid beetles consumed many common pest insects including eggs and larvae of: cabbage root fly (*Delia radicum*); Colorado
potato beetle (*Leptinotarsa decemlineata*); cereal leaf beetle (*Oulema melanopus*); and Southern corn rootworm (*Diabrotica* spp.) (Kromp 1999). One example of a dietary specialist is the snail predator, *Scaphinotus* spp., which has an elongated head and prothorax. Similar morphological adaptations can be seen in the forelegs of *Loricera* spp., and allow for the capture of quick-moving springtail prey (Hexapoda: Collembola). In addition to invertebrate prey, omnivorous ground beetles consume plant matter including fruit (Lee 2001), pollen grains (Lundgren 2009), and weed seeds (Honek 2003).

**WEED SEED PREDATION**

Post-dispersal seed predation by ground beetles may be an important source of weed reduction in agricultural areas (Brust and House 1988, Westerman 2003) and many reviews explore the potential of exploiting this granivorous tendency as a means of biological weed control (Gallandt 2005, Mauchline 2004, Menalled 2000, Zhang 1997). Seed-eating beetles are especially common in the *Amara* and *Harpalus* genera. A field study from Maine indicated that over 11 days, 56% of placed seeds had been removed, and that 43% could be attributed to predation by invertebrates (Gallandt 2005). Weed seed predation by carabids is dependent on various factors including: inherent seed properties, seasonal availability, and location of seeds relative to the soil surface.

Physical seed characteristics influence predation. In a recent study, Lundgren *et al.* used a plate compression tester to determine the relative compressive yield strength (CYS) of various seeds. *Amaranthus retroflexus* and *Chenopodium album* have seed coat strengths of 47.8 MPa and 24.4 MPa, respectively, and have identical densities of 1563 mg/cm$^3$. For comparison, broccoli, (*Brassica oleracea*) has a CYS of 3.65 MPa and a density of 1.128 mg/cm$^3$. Free-choice feeding trials with the two weed species indicated a significant
preference for harder ($F=11.07$, $p=.01$), more dense ($F=7.20$, $p=.03$) seeds by *Harpalus pensylvanicus* (Lundgren 2007).

The ratio of seed size to beetle size was studied by Honěk *et al.* (2003). They found that smaller carabids (<5mm) prefer smaller weed seeds like those of *Cirsium arvense* and *Capsella bursa-pastoris*. Similarly, in a confined terrarium experiment, *H. pensylvanicus*, a relatively large carabid, consumed an average of four large crabgrass seeds per day, and zero smaller chickweed seeds. Conversely, the smaller-bodied *Amara cupreolata* preferred chickweed to crabgrass by 48% (Barney 1986).

Availability of seed likely influences predation rates. Previous literature states that consumption and preferences of carabid beetles is influenced by temporal variation (Hulme 1998, Saska 2004), and it is possible that co-evolution mediates these trends. A recent laboratory experiment revealed that granivorous beetles strongly preferred freshly shed *Taraxacum officinale* seed versus buried (Martinoková 2006). The authors propose that since dandelion seed degrades shortly after seed shed, there is a limited window of availability for carabids to consume it. Similarly, removal of *Taraxacum inodorum* seed was greatest in late June, which coincides with the peak mating season for *Harpalus affinis*, a spring-breeding granivorous carabid (Martinková 2006). On the other hand, seed predation in late summer through autumn is correlated with increased activity of fall-breeding species like *Pterostichus melanarius* (Westerman 2003). Biological synchronicities between weed seed shed and peak carabid activity are probable, yet rarely studied in the context of agricultural fields.

In addition to temporal availability of seeds, post-dispersal seed predation is dependent on spatial availability of the seed to the herbivore. The common perception is that buried seed is unavailable to predation by granivorous insects (Thompson 1987).
Positions of weed seeds relative to the soil surface differ depending on tillage regimes (Gallandt 2006, Peachey 2005). In conventional tillage, there is a great amount of vertical seed movement (Benvenuti 2007), and seeds are either buried or brought to the surface, depending on which year the tillage is performed. Conversely, no-till areas tend to leave seeds on the surface (Teasdale 1991), where they may be more accessible to granivores such as carabid beetles (Harper 1977, Menalled 2007). Differences in seed location as a consequence of tillage may greatly influence preferences and removal rates of weed seeds by invertebrates.

Weed seed consumption rates are difficult to estimate in field situations due to trophic interactions (Frank 2007). For example, in a recent study from New Zealand, researchers placed experimental trays of Chenopodium album and Veronica persica seeds in grass fields and discovered, via video monitoring, that 95% of seed loss was due to rodents and birds (Navntoft 2009). Similarly, a UK study found that seed predation by invertebrate herbivores was “negligible” (Hulme 1998). Therefore, seed removal studies are often done using caged platters that eliminate rodent or bird interference. This method helps provide a more accurate estimate of seed removal by invertebrates and carabid beetles are not hesitant to enter caged feeding stations (Crawley 1992). Other invertebrates (ants, slugs, earthworms) also contribute to post-dispersal seed loss. Opinions differ as to whether earthworms actually consume weed seeds (Eisenhauer 2009) or if dispersal is merely an unintentional consequence of translocation (Pollack 2007).

**EFFECTS OF TILLAGE**

Intensive primary tillage may be detrimental to carabid survival (Lys 1994, House & All 1981). A Maine study found that carabid activity-density was reduced 54% after
moldboard plowing (Hatten 2007). Since carabids overwinter in the soil, there may be direct mortality caused by physical damage to the sensitive larval life stage, especially with spring tillage. For instance, a study from Ireland examined *Pterostichus melanarius* females for the presence of eggs and fat bodies as a measure of age to determine if females had newly emerged or if they were mature egg-layers, and they were unable to find any newly hatched beetles in fields that had been exposed to conventional primary tillage (Fadl, 1996).

Conventional tillage (CT) may elicit different responses from spring vs. fall-breeding carabids due to synchronicities between tillage timing and larval development. Species diversity as well as total number of carabids may be up to seven times greater in systems with reduced tillage (Carmona and Landis 1999, House 1983). Granivorous species, in particular, seem to exhibit preference for undisturbed areas (Menalled 2001, Tonhasca 1993).

Eliminating tillage is commonly done to preserve soil quality and reduce erosion (Little 1987) and it may lend better habitat structure (Kromp 1999) and prey availability (Lee 2001, Magura 2004) for beneficial ground beetles. Environmental aspects such as humidity and microclimate are also altered in no-till (NT) systems (Clark 1995). Often, NT management involves other cultural weed control techniques such as crop rotation and the use of cover crops. Cover crop residue adds a significant amount of protective habitat for ground beetles, up to 12,000 kg/ha of organic matter (House 1983). Planting leguminous cover crops can help improve soil conditions due to associations with nitrogen-fixing bacteria (Sylvia 2005). Another benefit of cover crops is that some of them have allelopathic qualities which can help suppress weeds (Gallandt 2006). These practices, in addition to reduced pesticide use, may help promote and maintain ground beetle populations (Brust and House 1988, House and Stinner 1983, Lys 1994).
Another topic of research is the effectiveness of using refuge strips as habitat for carabid beetles. Often called beetle-banks, artificial habitat is provided by growing various flowering plants in rows between and around the cash crop. There are reports of increased activity-density (Carmona and Landis 1999) and diversity (Lundgren 2006) of ground beetles in refuge strips. More specifically, granivorous species like *Harpalus pensylvanicus* and *Anisodactylus sanctae Crucis* are more common in refuge areas (Menalled 2007). In one study, landscape context and amount of bordering grassland was a more important factor for abundance of spring-breeding carabids than whether a farm was organic or conventionally managed (Purtauf 2005). Transect data from a recent study suggest that movement of carabids occurred from field edges toward the center (Nash 2008), which supports the theory that biologically-diverse areas adjacent to arable land help to harbor and maintain the presence of beneficial ground beetles. Refuge strips and reduced-tillage areas are touted as enhancing carabid abundance and diversity likely due to a combination of factors including: sampling methodology, physical protection from climate and predators, and an increase in availability of both floral and faunal prey. However, many of these studies are conducted on Long-Term-Ecological-Research (LTER) land, or sampling observations are made during one season of one year. In both cases, estimates of carabid activity-density may not reflect the changing landscape associated with production agriculture and vegetable cropping systems.

Another problem with refuge studies is that beetles are free to move from one habitat to another, and it is not clear whether refuges are acting as a biological source or as a sink (Corbett and Plant in Carmona and Landis 1999). Very few ground beetle studies attempt to limit or reduce carabid movement between experimental treatment areas. Although this method is extremely labor intensive for large-scale studies, it may be crucial for
determining patterns that would otherwise go undetected. As mentioned, trapping methodology can be greatly influenced by abiotic factors such as density of the surrounding vegetation (Greenslade 1964) and amount and intensity of light that reaches the soil surface (Purtauf 2005). A myriad of studies observe lower pitfall catches in places with a large amount of plant cover (Lee 2001, Carmona 1999, Lys 1994). Others find that activity-density (AD) is greater in highly vegetated areas (Menalled 2007, House 1983). One way to address these discrepancies is to measure other response variables, in addition activity-density (AD). If AD studies are combined with an estimate of some other life process (e.g. fecundity, consumption, etc.), it becomes easier to accept or refute the notion that refuge areas are beneficial for ground beetles.

EFFECTS OF PESTICIDE

Many insecticides currently used in crop production are non-selective and thus may pose serious risks to non-target organisms (NTOs). Carbamates and organophosphates, in particular, have high acute toxicity and are usually applied at heavy rates (1-2 kg a.i./ha). A common conception is that broad-spectrum insecticides have a detrimental effect on carabids. One study notes that as parathion application increased from 6-100% of UK wheat fields, corresponding carabid catches and health were dramatically reduced; *Pterostichus melanarius* levels declined 81% and 95% for pitfall and quadrant sampling, respectively (Kromp 1999). Also, biomass of the captured beetles was reduced by 90% compared to pre-spray years, indicating a significant effect on overall beetle health (Kromp 1999). A recent study conducted by Nash (2008) examined beneficial predator response to tillage and insecticide in canola and cereal crops across Victoria, Australia. *Notonomus gravis* was the dominant carabid species in his study and comprised, on average, 78% of the total carabid
community. *Notonomus gravis* is a generalist predator with a life history very similar to that of *Pterostichus melanarius*. Nash found that insecticide use and *N. gravis* activity density were negatively correlated (Spearman’s r = -0.582, p≤0.01).

Interestingly, there are also cases of an increase in beetle activity-density after an insecticide application. This could be due to the presence of refuge habitats that provide a sort of buffer, either via vegetation (Lee 2001) or soil structure (Sylvia 2005). It is also possible that a false positive effect is evident because beetles are searching for prey. That is, it could be the ‘activity’ component of activity-density that causes apparent differences. This was quantified by Chiverton et. al (1984), who examined gut-fullness and the amount of solid food present in *P. melanarius* beetles from spring barley plots either treated or untreated with two broad-spectrum insecticides. He found that in untreated plots, the number of gravid females with completely full guts was 28 versus only 5 and 14 from fenitrothion and sumicidin treated plots, respectively. Others agree that apparent increases in trap catches after an insecticide spray is most likely due to indirect biotic changes such as food availability and decreased competition (Lee 2001, Prafsika 2008).

Carabid beetles are generalist predators that form a link in the soil food web. As such, they have been shown to be sensitive to pesticides aimed at other organisms. Langan et al. (2004) found that when *P. melanarius* beetles were fed slugs that had been killed by the mollusicide methiocarb, longevity was reduced 85% compared to either the control or to slugs that had been killed by the less-toxic alkaloid metaldehyde. The same study found that feeding duration was actually greater on slugs that had been contaminated with metaldehyde than on uncontaminated prey (H=7.49, p=0.02) (Langan 2004).
Another intriguing proposition is that certain carabids may be developing resistance to pesticides. An extensive review by Frietag (1976) has some valuable, albeit dated, information about the relationship between pesticide use and carabids. It has been suggested that *P. melanarius* is tolerant enough to withstand leptoephos and chlorfenvinphos, even at levels toxic to Lepidopteran pests (Tomlin 1975 in Frietag 1976). Sub-lethal pesticide doses can influence carabid beetle behavior, as well. When Prafsika (2008) exposed *Scarites quadriceps* beetles to varying levels of insecticide-treated filter paper, beetle activity and step-ratios of movement showed dose-dependent increases. The authors attributed this phenomenon to hormesis, a positive biological response to a seemingly negative input. However, both the Tomlin and Prafsika experiments were performed within a laboratory and one should take caution before extrapolating their results to a field environment. By evaluating the effects of insecticides on carabids using a Potter spray tower, researchers can discern toxicity levels which can then be modeled to predict field responses.

In addition to insecticide trials, there are studies that report on the effects of herbicides on carabid beetles. A study examining the effect of herbicides on avian habitat and prey selection found that number and dry weight of arthropod species were greatest in plots untreated with bromoxynil and imazamethabenz (Taylor 2006). Untreated plots had more carabids compared to either a monoculture of spring wheat, or an intermediate area of dryland cereals mixed with weeds (Taylor 2006). The same study found that plot treatment was highly significant (*F*=9.0, *p*=0.02) for *Pterostichus* species in particular, and that weedy areas had almost twice the abundance of medium sized carabids, as measured by pitfall traps. The consensus of this study is that any change in arthropod activity-density after an herbicide treatment can likely be attributed to changes in the plant community structure.
WEED RECRUITMENT

The final component to this study is to review how annual weeds are influenced by different tillage regimes. Each year, weed seeds accumulate in the soil, and they can remain viable there for well over 100 years (Thompson 1987). Thus, most weed control programs focus on ways to reduce the accumulation of seeds in the soil, which is commonly referred to as the soil seedbank (Swanton and Booth 2004). Seed shed is the main cause of increase to the soil seedbank (Harper 1977) and is a determining factor of weed population potential (Menalled et al. 2001). Seed shed timing, amount, and percentage of viable seeds are all important considerations for management and can vary widely between species. For instance, in one year, seed density of redroot pigweed in a Minnesota corn field was estimated at approx. 1780 seeds/m² and viable seed accounted for 11% of that. However, the next year, seed shed was reduced to 1250 seeds/m² but 59% of it was viable (Forcella et al. 1996). Forcella also reported that wild-proso millet may produce up to 557 seeds/m² and viability can be as high as 76%.

Opinions vary as to whether no-till systems increase or decrease the emergence of summer annual weeds. Some propose that reduced tillage limits vertical movement of weed seeds and the resulting accumulation of seeds at the surface causes high weed density the following year (Benvenuti 2007, Teasdale 1991). However, other studies report lower weed densities in reduced-tillage systems. Peachey et al. found that a continuous system of direct-seeding in the spring in a snap bean-sweet corn-wheat rotation reduced overall weed density by 63-86% (2006).

In addition to tillage intensity, the timing of soil disturbance may be important for controlling summer annual weeds. For example, changing from a continuously
conventionally-tilled system to a reduced tillage protocol caused density of hairy nightshade to be reduced from 410 to 70/m², when averaged across herbicide levels (Peachey 2006). However, the same weed dramatically increased (410 to 770 plants/m²) when it was spring tillage that was reduced (Peachey 2006), which suggests that reducing tillage can yield different effects on the same weed depending on which season it is performed. Also, weeds may respond to tillage differently based on taxonomic biology. One study found that no-till systems showed an increase in monocot species such as crabgrass and goosegrass, where as density of dicot weeds, particularly common lambsquarters (Chenopodium album), was higher in conventionally-tilled plots (Teasdale 1991). Reducing tillage may lead to weed community shifts (Thompson 1987) and repeated management selects for weeds that are particularly well-adapted to low-input environments. Thus, tillage regimes can be manipulated to provide the best possible control of various species. It is essential to understand how cultural control tactics, such as reducing tillage, can be integrated into a weed management program.
Chapter 2 - Evaluating the Effects of Agricultural Disturbance on Ground Beetle (Coleoptera: Carabidae) Activity-Density and Weed Seed Predation

ABSTRACT

Conservation of natural enemies is an important component of sustainable agriculture. Ground beetles (Coleoptera: Carabidae) are beneficial insects that prey on a wide variety of agronomic pests (Symondson 2002, Kromp 1999). Carabids may also help contribute to integrated weed management goals by reducing the amount of weed seeds left on the soil surface (Menalled 2007, Lundgren 2006). Our research sought to quantify how typical farming practices such as tillage and broad-spectrum insecticide use affect beneficial ground beetle assemblages and weed seed predation potential in vegetable row crop settings. Experimental plots were defined using plastic landscape fencing to limit beetle movement, and extensive sampling provided the opportunity to evaluate overall response to disturbance as well as seasonal activity trends in a changing landscape. Weed seed removal was estimated using caged platters placed in the field. Results indicate that AD increased greatly in the years following initial field disturbance. Main effects of plot management elicited different effects on different carabid genera and significance varied by year and site. We demonstrate that AD of a generalist predator, Pterostichus melanarius (Ill.) increased after repeated application of broad-spectrum insecticides. Data from seed stations suggest that seed loss was highly variable between years and may have been influenced by temporal trends as well as landscape and habitat.

KEYWORDS: Carabidae, agro-ecology, post-dispersal seed predation, conservation tillage, biological weed management. ABBREVIATIONS: Amaranthus powellii (AMAPO), Solanum sarrachoides (SOLSA), Panicum miliaceum (PANMI).
INTRODUCTION

Carabid beetles (Coleoptera: Carabidae) are beneficial, generalist predators frequently found in agricultural settings throughout the world. They are known to reduce populations of various detrimental invertebrate pests including aphids (Kromp 1999, Symondson 2002), slugs (Langan 2004, Fawki 2005), and black vine weevil larvae (Lee 2001). Additionally, omnivorous ground beetle species consume weed seeds as part of their diet (Zhang 1997, Saska 2004). Recently, researchers have been evaluating the granivorous habits of ground beetles as a potential means for managing weed populations in agroecosystems (Westerman 2003, Lundgren 2007, Menalled 2007).

A variety of forces shape the biology of agroecosystems. Pesticides such as insecticides and herbicides are nearly essential tools for pest management in many cropping systems. In fact, agricultural pesticides account for over 77% of total pesticide sales in the United States, with annual revenue surpassing $181M (Pedigo and Rice 2006). However, based on current energy trends and consumer demand for environmentally-sound farming techniques (Uri 2000), there is a growing need to develop more integrated pest management programs. Incorporating other, non-chemical management tactics such as cultural and biological control is one way to meet these rising demands. These two strategies of alternative weed management are well researched in their own right, but rarely do studies focus on the combination and possible synergism of the disciplines. There is relatively little published on abundance, diversity, and dietary preferences of Carabidae in Oregon (Kavanaugh 2008). With mild, wet winters and short dry summers, the Pacific Northwest offers a unique climatic opportunity to evaluate ground beetle assemblages and how they
might be influenced by common crop management practices such as tillage and insecticide use.

Cultural control, such as reducing tillage, is commonly used in integrated pest management, and many studies report on the effects of highly vegetated areas on carabid community dynamics (Carmona and Landis 1999, Lys 1994, Westerman 2003). Since carabids are opportunistic predators, providing habitat via areas of reduced tillage might help to reduce populations of crop pests. Research shows that species diversity as well as total number of Carabidae may be up to seven times greater in no-till (NT) cereal fields (Brust & Stinner 1983, Menalled 2007). Additionally, low-disturbance areas are touted as harboring a greater abundance of granivorous ground beetles (Menalled 2001, Lundren 2006), and that these beetles may contribute an ecosystem service as biological weed control agents (Zhang 1997, Westerman 2003).

Despite the perceived benefits, eliminating tillage completely (no-till, NT), is not widely accepted or practiced in moist environments such as the Willamette Valley of Oregon. Environmental constraints (i.e. long, wet spring seasons) necessitate at least minimal pre-plant tillage. However, one option currently available to vegetable growers is to perform strip-tillage (ST), which allows for seed rows to be cultivated before planting, but leaves between-row areas undisturbed (FIG. 1). Little is known about how reduced tillage (ST) cropping affects carabid beetles in the Pacific Northwest.

The effect of broad-spectrum insecticide use on carabid beetles is a well-studied issue with mixed results. Some field studies suggest that abundance of beneficial carabids is negatively affected by insecticide (Westerman 2003, Nash 2008) while others (Lee 2001, Chiverton 1984) state that Carabidae activity-density increases after application of broad-
spectrum insecticides. Clearly, there is a need for continued investigation into how broad-spectrum insecticides affect beneficial, non-target organisms, such as carabid beetles.

Change in carabid activity-density (AD) is an interesting aspect of ecological studies, but public interest in the topic will be greatly enhanced if increased AD can be demonstrated to translate into a specific ecosystem service, such as removal of weed seeds from agricultural fields. Studies from the Midwestern United States (Menalled 2007, Carmona 1999), as well as Europe (Honek 2006, Hulme 1984) and New Zealand (Navntoft 2006) have attempted to relate levels of ground beetle activity-density with seed loss from field experiments. However, seed removal is not always directly correlated with activity-density (Lundgren 2006, Frank 2007) and results are often specific to region, crop, or study species. Moreover, experimental methods tend to vary widely throughout the literature and thus contribute to the uncertainty of quantifiable seed predation rates by invertebrate herbivores. Abundant differences and apparent in situ factors warrant the continuation of research into how ground beetle AD relates to weed seed predation in agricultural settings.

The objectives of this project were to determine: a) the effects of tillage and broad-spectrum insecticides on ground beetle assemblages; and b) how changes in land management might influence the weed seed predation potential of these beneficial arthropods. Our main hypotheses were that disturbance would have a negative effect on carabids, and that areas of repeated conventional tillage would not harbor as many ground beetles as reduced-tillage areas. Use of a broad-spectrum insecticide was expected to be detrimental to carabids, but we were unsure of the long-term effects, if any. Finally, we expected that weed seed removal would be greater in areas of reduced disturbance, due to the possible increase in carabid abundance.
MATERIALS & METHODS

EAST SITE

Research was conducted on a 2.5A parcel on the eastern edge of the OSU Vegetable Research Farm (Linn Co., OR). Prior to the experiment, the land had been in a tall fescue rotation for over a decade and was relatively free of broadleaf weeds. Soil at the site has been classified as Chehalis silt loam (40-40-20). In June of 2007, fescue was sprayed down with glyphosate at a rate 2.2 kg/ha. Experimental plots were defined and measured 10m by 20m each, separated by 6m alleys. Environmental data were collected using a soil probe to measure temperature at the soil surface and 2.5cm below. The effect of plant canopy density on light interception at the soil surface was estimated throughout the experiment using an AccuPAR LP-80 Leaf Area Index (LAI) ceptometer (Decagon Devices Inc., Pullman, WA.).

The East site experiment began in 2007. Project design was a RCB split-plot, $3^2$ factorial with 6 replications. Main factors imposed were: spring tillage (SPR); use of a broad-spectrum insecticide (INS); and fall tillage (FALL). Year 2 (2008) was an assay year; there were no direct treatments applied in year 2 and all plots were managed the same. In Year 3 of the experiment, (2009) spring tillage and broad-spectrum insecticide treatments were again applied to the same plots as in 2007. Carabid beetles were sampled in all three years via pitfall trapping to provide an estimate of response to: initial treatments (year 1); management of the previous season (year 2); and repeated, cumulative input (year 3).

PLOT MANAGEMENT

Spring soil disturbance was the first imposed factor and consisted of two levels; conventional tillage (CT) and strip tillage (ST). During the week of 16 June, 2007, CT plots
were disked twice with a 45cm disc plough and followed by two passes with a rotary vertical line tiller. Reduced primary tillage (ST) was done with a segmented roto-tiller, which resulted in 0.45m of undisturbed soil between planting rows (FIG. 1). All plots were planted to Phaseolus vulgaris var. Savannah (Harris Moran, Modesto, CA.) on 22 June 2007 at a rate of 20 seeds/m using a Max Emerge® planter (John Deere Corp.). Black plastic landscape fencing (15cm width, Valley View Inc.) was installed at the perimeter of each plot so that 2/3 of the fencing was buried, and approx. 5cm was left above ground. This created a barrier around each plot, which helped to define the treatment area and limit beetle movement.

At first trifoliate of snap bean growth (11 July 2007), bifenthrin (2-Methyl-3-phenylphenyl)methyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate; Discipline 2EC) was applied to randomly designated plots (INS+) at a rate of 0.021 kg/ha. Bifenthrin is a broad-spectrum pyrethroid insecticide commonly used in orchards and row crops. It has a high \( K_{oc} \) (1.31-3.02x10^5), indicating strong soil adsorption, and stated half-life in soil is >300 days (Fecko 1999). Bifenthrin was applied with water at 186 L/ha with a CO\(_2\) powered backpack sprayer using a 305cm hand-held boom with 6-XR8003 nozzles set 51cm apart. The bifenthrin application was intended as an arthropod exclusion, but post-spray pitfall sampling suggested that it was not reducing arthropod densities and so on 3 August, ethoprop (1-(ethoxy-propylsulfanylphosphoryl)sulfanylpropane), Bayer Corp, Mocap 6EC™) was applied. Ethoprop was applied at 9.6 L/ha using a hand wand equipped with an XR8003 nozzle directed between each row and below the snap bean canopy so that the soil surface was completely covered with spray but foliage was not damaged by the ethoprop. Irrigation was applied immediately after the ethoprop to incorporate the insecticide into the soil.
Figure 1 - Conservation tillage, often called strip-tillage (ST) leaves visible strips of residue in the field. This type of intermediate tillage helps reduce erosion and maintain soil quality, while still allowing pre-plant cultivation in crop rows. Additionally, ST may provide habitat for beneficial ground beetles.
In September of 2007, the snap beans were flailed, main plots split, and half of each randomly selected plot was conventionally-tilled to prepare a seedbed for the cover crop. The other half of each main plot was not tilled. Landscape fencing was re-installed after tillage as described above, resulting in 48 – 120 m² sub-plots. The entire experiment was seeded with a cover crop mixture of 80:50 *Hordeum vulgare* var. Steptoe and *Vicia sativa*, at a rate of 110kg/ha. The cover crop grew through the winter and required no irrigation due to the wet winter climate in Oregon. In late April 2008, cover crop biomass was sampled randomly from each end of each subplot (n=96) using a 0.25m² PVC sampling square. All above ground plant material was cut, placed into paper bags, and dried at 40°C for 60 hours. Plant matter was separated by species and dry weights were recorded.

*Cucurbita maxima* var. Golden Delicious winter processing squash was planted 2 June 2008 into each plot at a rate of 2.7 seeds/m² using a cross-slot planter. All squash was direct-seeded, meaning it was planted into the remaining cover crop residue without first tilling the soil to prepare a seedbed. A 12-29-10 NPK fertilizer was banded next to the seed row at planting at 420 kg/ha. Squash was harvested and yield data were taken on 16 October, 2008. This concluded activity for year 2 at the East site.

On 2 June 2009 (year 3), plastic fencing was removed from each end of each plot to allow for plots to be flailed. CT and ST tillage treatments were again applied to the same plots as described for the snap bean planting in 2007 (year 1). Plots were planted to *Phaseolus vulgaris* var. Savannah (Harris Moran, Modesto, CA) at a rate of 20 seeds/m of row. NPK fertilizer (16-16-16) was banded next to each row at planting and the crop was irrigated throughout the growing season at approximately 3cm of water per week. Landscape fencing was reinstalled the week of 8 June 2009, and left in place until 24
September 2009. Ethoprop insecticide was applied with water at 186 L/ha using a CO$_2$
powered backpack sprayer and a 305cm hand-held boom equipped with 6-XR8003 nozzles
set 51cm apart. The 2009 ethoprop application was done after snap-bean planting to the
same plots and at the same rate as in 2007. Irrigation was applied during and immediately
after the application to incorporate the insecticide.

A summary of main effect methodology can be seen in figure 2. The experiment ran
for 3 consecutive years, beginning in 2007 with the application of main factors (spring tillage,
insecticide, fall tillage), each with two levels. The resulting eight treatment combinations
provided us with a management continuum along which to measure ground beetle activity-
density and weed seed predation. Management of each plot either tended towards a high-
input scenario, common in large scale production agriculture, or a reduced-disturbance
system more common in low-impact farming (FIG.2).

GROUND BEETLE SAMPLING

Pitfall cups were installed in each plot to estimate activity-density (AD) of carabid
beetles. The pitfalls were made of two nested plastic cups (10cm diameter) set even with
the soil surface. In 2007, there were three pitfalls per main plot (east, west, center (n= 72)),
and data were collected from 9 July - 30 September, over five to eight nights. All totals were
divided by number of nights left open, thus normalizing each collection event to the average
number of beetles per pitfall per night (BPN). Pitfalls were closed between sampling periods
by either placing a plastic lid on, or inverting, the inner cup. Sampling efforts increased over
the next two years (n=96) and ran from 24 May to 1 October 2008 and 24 April to 24
September 2009. Beetles were site-identified to at least genus level, and re-released in the
same subplot. Random sub-samples of the most abundant beetles were taken to the lab and
identified to species using taxonomic keys (Lindroth 1961, LeBonte unpubl.). In addition to area-wide sampling, 1.5m\(^2\) galvanized metal arenas were installed in 12 of the 24 plots to estimate the absolute-density of ground beetles in a confined area. Arenas were set at random locations in each plot, and sunk in to a depth of approx. 10cm. The metal arenas were placed on 16 June 2007 and removed on 15 August 2007.

Figure 2 - Seasonal methodology of factorial treatments resulting in 8 experimental combinations (A-H). These represent a continuum of disturbance ranging from low (bottom path), to high (top path). All treatments seen here were applied during Year 1 (2007) at the East site. Spring and summer treatments were repeated in Year 3 (2009) at the East site and also Year 1 (2009) at the West site.
WEED SEED LOSS

In addition to directly measuring activity-density of carabids via pitfalls, seed removal was measured to estimate the effects of agricultural management on weed-seed predation potential. Seed loss was calculated by placing 2 seed stations in each plot (n=48 in year 1, n=96 in years 2 and 3). Seed stations were constructed by filling a 100mm x 15mm lid or base of a Petri dish to just below surface level with white plaster (DAP Products Inc., Baltimore, MD). Weed seed from the surrounding area was collected prior to each experimental year and thrashed to collect seed stock for platters. Individual seeds were placed on the surface of the plaster dish without any sort of adhesive. Number of weed seeds varied by year and site and consisted of either pigweed (Amaranthus powelli - AMAPO), hairy nightshade (Solanum sarrachoides – SOLSA) or wild-proso millet (Panicum miliaceum – PANMI) offered alone (30-60 seeds) or together (10 seeds per species) (See TBL. 2.1 for details).

Wire screening (1.5cm opening) was cut into 15cm wide pieces, formed into a circle, and placed around each platter. Additionally, finer mesh (1mm opening) was wrapped around the metal screening for half of the platters (n=48), to prevent carabids from entering. Therefore, at each sampling location, one caged platter was intended to allow access by arthropods (ARTH), and one served as a per-site control (TOTAL). Seed loss was calculated by subtracting the number of seeds missing from TOTAL cages from the number of seeds removed from ARTH cages. In this way, calculated seed loss was most likely due to removal by invertebrates. Each caged platter was topped with a 12cm PVC cap and the entire apparatus was covered by a molded 0.17m² piece of plexi-glass plastic to reduce interference by rain and irrigation. Seed loss was measured in each of the three years of the experiment.
at the East site (TBL. 2.1). Additionally, preliminary seed loss data was collected during June 2006 at the East site location and the field adjacent to the North. Both 2006 fields were planted to tall fescue at the time and provided baseline estimates of seed removal rates before the experiment officially began in 2007.

**WEST SITE**

The first two years of the experiment were repeated in 2009 and 2010 at a different site on the OSU Vegetable Research Farm (Linn Co., OR). The second field site was located on the western edge of the farm. Soil type and overall project design were the same as the East site. Previous land management was different at the West site; the experiment was placed on an area that had been in constant vegetable rotation for almost 10 years, and therefore, the amount of weed seed already present in the soil was much greater than at the East site. The year prior to starting the experiment, the West site was utilized for a trial examining the effects of buried *Sclerotinia* on snap bean disease expression (Stone & Mikazoe 2008, in press). Proximity to landscape features was also different at the second location. Approximately 6m to the West, and just across a chain-link fence, was a heavily managed golf course and a small run-off tributary of the Willamette River. There were minor methodological differences between the two sites.

At the West site, plots were again surrounded by plastic landscape fencing but plot size was reduced to 11m x 9m, and arranged in five blocks, yielding 20 - 100m$^2$ main plots. Spring tillage (CT/ST) was performed during the week of 13 June 2009, and plots were planted to snap beans (*Phaseolus vulgaris* var. Savannah) at the same rates and methodology as the East site. Ethoprop (1-(ethoxy-propyl)sulfanylsulfonylphosphoryl)sulfanylsulfanylpropane), Bayer Corp., Mocap 6EC™) insecticide was applied after planting (17 June 2009) to designated plots
(INS+) at the same rate as in years 1 and 3 at the East site (9.6L/ha) and with the same application and irrigation methods.

A barley and vetch cover crop (80:50, 150kg/ha) was planted in September 2009 and sprayed down by applying glyphosate at 2.2 kg/ha on 26 April 2010. A second application of glyphosate was applied approx. one month later to control vetch that was not controlled by the earlier treatment. Plots were strip-tilled once on 1 July 2010 and corn (Zea mays var. Coho) was planted the following day at a rate of 20 seeds/m using a John Deere MaxEmerge™ planter. A 16-16-16 NPK fertilizer was banded next to the row during planting.

Activity-density sampling of carabids at the West site consisted of 2 pitfalls per 100m² plot, arranged in a semi-random grid pattern. In year 1, collection events ran from 6 July to 26 September 2009 and total beetles were divided by nights left open to get a standardized measure of beetles per pitfall per night (BPN). Year 2 AD samples were taken from 9 June to 9 September 2010.

Seed stations at the West site consisted of plaster-filled Petri dishes (100 x 15mm) surrounded by wire mesh and screen to either allow (ARTH), or exclude (TOTAL) access by arthropods. Additionally, a 30cm square piece of 1mm fine mesh screening was placed under each seed station to reduce interference by vertical-burrowing worms. Rain protection was provided by white plastic decorator pots (18.4cm diameter) (McConkey Co., Sumner WA) elevated 5cm off the soil surface, and placed over each caged platter. Seed stations were set with the same three species used at the East site: pigweed (Amaranthus powelli - AMAPO), hairy nightshade (Solanum sarrachoides – SOLSA) and wild-proso millet (Panicum miliaceum – PANMI) (TBL.2.1).
Table 2.1 - Detailed methodology of seed station trials. Weed seeds offered were: *Amaranthus powellii* (AMAPO), *Panicum miliaceum* (PANMI), and *Solanum sarrachoides* (SOLSA). N=number of paired seed stations (either allowing or excluding access by invertebrates). Location “Grove” was located adjacent north of the East site, and was sampled only as a preliminary baseline.

<table>
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<th>YEAR</th>
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<th>SEED SPECIES</th>
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<td>8-Jun</td>
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<td>7-Sep</td>
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<td>10 ea.</td>
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</tbody>
</table>
RESULTS

SECTION A: Activity-Density Patterns

EAST SITE

Intensive random sampling over three years at this site provided an opportunity to examine not only treatment effects on carabid activity-density (AD), but also yearly variation and seasonal activity trends. At the East site, total trap captures of carabids increased as the study site transitioned from grassland to a managed vegetable cropping rotation. There were 150 total carabids collected in pitfall traps in year 1 (2007), 1166 in year 2 (2008), and 5041 in year 3 (2009). However, sampling efforts were not consistent between years, and therefore are interpreted on a year-by-year basis.

Diversity of carabid fauna was relatively low at the East site. In fact, less than one dozen species were consistently found in pitfall traps and there was strong evidence of species dominance. *Pterostichus melanarius* (Illiger), a generalist predator abundant in arable land (Thomas 1998, Fadl 1996, Langan 2004) was the most common beetle found in our study. *P. melanarius* is a fall-breeding species and accounted for over 70% of the sampled community in each of the three years. The next most common genus of carabid at the East site was *Amara*. *Amara* spp. are generally diurnal spring-breeders and may be more granivorous than *Pterostichus* spp. (Honěk 2003). The usual peak activity period of spring-breeding carabids is from early March until early June, when overwintering adults emerge and are actively foraging and mating. Larvae develop quickly over the summer, and there may be another small activity peak as the new generation emerges from pupation. Indeed, we noted high populations of *Amara* spp. from May to June and AD declined with time. Similarly, literature classifies *P. melanarius* as a fall-breeder with activity highest in late
August, just before eggs are laid (Lindroth 1964). A second activity peak may sometimes be
evident as overwintering adults seek food reserves (Thiele 1977). We were able to confirm
this at the East site, when P. melanarius AD peaked at two different times in late summer to
early fall. *Anisodactylus binotatus* was the third most commonly sampled species at the East
site. Other ground beetle species collected included *Anisodactylus sanctaeclercus, Harpalus
affinis, Harpalus pensylvanicus, Agonum mulleri*, and *Nebria brevicollis*. Smaller fauna such
as *Loricera* spp. and *Bradycellus congener* also were present at the East site.

**YEAR 1 (2007)**

The confined metal arenas (n=12) in place from 17 July to 15 August in Year 1
estimated the absolute density of *P. melanarius* to be approximately 0.47 beetles/m². There
was no effect of either spring tillage (SPR) or broad-spectrum insecticide use (INS) on activity
density of *Amara* beetles, as measured by the confined arenas. There was, however, an
indication that the insecticide spray of year 1 reduced the density of *P. melanarius* within
arenas; the mean number trapped from areas that had been treated with bifenthrin and
ethoprop was 0.22 beetles/m² versus 0.71 beetles/m² from arenas placed in unsprayed plots
(SEM±0.09, F=2.84, p≤0.1).

Other pitfalls placed outside of the metal arenas also suggested that broad-spectrum
insecticide use reduced activity-density (AD) of *P. melanarius* in year 1. Average number of
beetles per pitfall per night (BPN) was 0.07 in untreated plots and 0.04 in plots that had been
treated with insecticide (SEM±0.01, F=2.44, p≤0.1), when averaged across the season.
Repeated measures analysis indicated that significance of main effects on *P. melanarius* AD
changed with time. For example, in the first collection event (9-17July), average beetles per
night (BPN) was 1.1, 100% of which were sampled from strip-tilled plots (F=3.14). Spring
tillage continued to be a significant factor affecting *P. melanarius* AD in collection event 2, when beetles averaged 0.10 and 0.03 BPN from conventional and strip-tilled plots, respectively. Effect of insecticide was most evident during the third collection event (1-8Aug 2007), and there were more *P. melanarius* collected from untreated plots (SEM± 0.02, F=5.44, p≤0.01). There was no evidence of main effects on trap catches of *Amara* beetles in 2007, likely due to the very low sample size of that genus during Year 1 (n=6).

**YEAR 2 (2008)**

Year 2 of the experiment served as an assay year; no treatments were applied. In this way, we were able to measure how soil disturbance and inputs from the previous growing season influenced carabid activity-density the following year. Recall the split-plot nature of this study, which effectively increased plot number (n=24 to n=48) and reduced plot size from 240 m² to 120 m² in September 2007. The average number of beetles per pitfall per night (BPN) in year 2 was 0.41, and activity peaked to 0.78 mean BPN around mid August (TBL. 2.2). Mean number of total *P. melanarius* trapped within insecticide-treated plots was 10.3, versus 8.6 from untreated plots (F₁,₁=1.26, p=0.27). There was no evidence of the year 1 insecticide applications affecting *Amara* AD in year 2 (F₁,₁=0.02, p=0.90). This is not entirely surprising, considering the flight and emigration capabilities of *Amara* beetles. A main effect of spring tillage was not evident during any time in year 2 (TBL 2.2). There was, however, a fall tillage effect on carabid AD during the first sampling period of year 2 (19 June 2008). Average BPN at that time was 0.28 in plots that were direct-seeded (nt) and 0.52 in plots receiving conventional fall tillage (ct) (F=4.17/p=0.05). There were no treatments applied in year 2, and also no detectable difference between means of overall carabid AD in treated (INS+) vs. untreated (INS-) plots for 8 of the 9 collection events (CE). However, the
AD sample taken 4 Aug 2008 (CE6) had an average of 0.51 BPN in plots that were not treated with insecticide the year before, and 0.75 BPN when insecticide was applied in year 1 ($F_{1,1}=8.05$, $p=0.04$). We were not expecting to see a residual effect of the previous year’s insecticide application. It is possible that the insecticide spray of year 1 limited invertebrate prey emergence in year 2, causing a period of increased activity by predacious carabids.

**YEAR 3 (2009)**

Spring tillage and insecticide treatments were re-applied in Year 3 and pitfall data were collected to measure the cumulative effects of spring tillage and insecticide on carabid assemblages. Sampling efforts were identical in years 2 and 3 and so direct comparisons of means are possible (TBL. 2.2, FIG.4). Mean number of total beetles per pitfall per night (BPN) was 0.57 in year 3, averaged across time and treatment. There was a strong effect of blocking, with the highest trap catches occurring on the Western most edge of the field (0.95 mean BPN in blocks I and II), and lower on the East end (0.55 mean BPN in blocks V and VI) ($SE\pm0.09$, $F=5.54$, $p\leq0.005$). Overall, activity-density was greater in insecticide-treated plots (0.80 BPN) vs. non-treated plots (0.63 BPN) in year 3. Figure 4 shows how the effect of broad spectrum insecticide differs between the assay year (2008, no treatments applied) and year 3 (2009, original treatments re-applied) of a cropping system. The effect of insecticide use varies with time in both years. The arrow in figure 4 indicates that insecticides were applied to 10 randomly selected plots between sampling events CE2 and CE3. For a brief period of time following the spray, trap catches are higher in INS- plots, which is in-line with our original hypotheses. However, in late July and August, AD is greatest in plots that were treated with insecticide (INS+).
Table 2.2 – ANOVA of effects of spring tillage (SPR) and insecticide (INS) on carabid AD in years 2 and 3 at the East site\(^a\). Treatments were applied in year 1 (2007) and again in year 3 (2009) to main plots. F-values indicated in bold are significant at \(p\leq 0.1\), alpha = 0.1. Mean BPN = beetles*pitfall\(^1\)*night\(^1\) (± SEM). CE= Collection event and sample date.

<table>
<thead>
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<th>CE6</th>
<th>CE7</th>
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<td>21-Jul-08</td>
<td>4-Aug-08</td>
<td>8-Aug-08</td>
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<td>1.11 (0.06)</td>
<td>1.23 (0.09)</td>
<td>0.47 (0.04)</td>
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\(^a\) back-transformed (\(\ln(x+1)\)) means of total carabids sampled
Figure 3 – Seasonal means of carabid AD vs. insecticide in years 2 (top) and 3 (bottom) at the East site. Asterisks over vertical bars indicate significance between means* at α=0.1 (*p≤0.1, **p≤0.05). Arrow represents when insecticides were applied. CE=Collection event, see text for details.

*back-transformed (ln(x+1)) means and standard errors.
WEST SITE

Overall, the carabid community at the West site was different than that at the East site. *P. melanarius* was the dominant species and there were very few *Amara* spp. sampled at the West location. Other abundant species were *Anisodactylus sanctaecrucis* and *Harpalus pensylvanicus*. As mentioned, both of these species are commonly found in agricultural fields, and the previous history of vegetable rotation at the West site may have been influential on shaping the carabid community. When averaged across the year 1 season (2009), there was a spring tillage by insecticide interaction for both male and female *Pterostichus melanarius* (TBL 2.3). The other most abundant taxa, as measured by pitfall traps, were unaffected by either spring tillage or insecticide in either year. Similar to the East site, main effects of spring tillage and insecticide applied in year 1 were only significant on *P. melanarius* catches for one trapping period (14 Aug 2009). Interestingly, peak carabid activity occurred 1 month later (BPN<sub>cec</sub>=2.11), but there was no evidence of treatment effects at that time (FIG. 4). There was no significance of main effect on carabid AD in the assay year (2010) at the West site (TBL 2.3). Thus, it appears that when disturbance is performed in year 1, the effect on carabid AD is only evident for the first cropping season. This is identical to what we saw at East site. Another similarity is that carabid overall abundance, as measured by pitfalls, increased greatly in just one year; mean BPN was 0.18 in year 1, and increased to 0.49 in year 2. Peak activity occurred during the same time each year (25-26 August), when there was an average of over 2 beetles per pitfall per night (TBL. 2.3). This suggests that overall, carabid AD was greater at the more heavily managed West site than at the relatively undisturbed East site.
Table 2.3 - ANOVA of effects of spring tillage (SPR) and insecticide (INS) on carabid AD in years 1 and 2 at the West site. Treatments were imposed in year 1. Means are from raw data, and represent the average number of beetles*pitfall\(^{-1}\)*night\(^{-1}\) = BPN.

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<th>MS</th>
<th>F</th>
<th>p</th>
<th>MS</th>
<th>F</th>
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<td>0.005</td>
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Ave. no. beetles (BPN)  0.21  0.16  0.00  0.01

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<th>F</th>
<th>p</th>
<th>MS</th>
<th>F</th>
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Ave. no. beetles (BPN)  0.71  0.27  0.43  0.08
Figure 4 – Seasonal means of carabid AD vs. insecticide in years 1 (top) and 2 (bottom) at the West site. Asterisks over vertical bars indicate significance between means at $\alpha=0.1$ (*$p \leq 0.1$, **$p \leq 0.05$). Arrow represents when insecticides were applied. CE=Collection event, see text for details. Activity-density (AD) back-transformed (ln(x+1)) means and standard errors (hash marks).
SECTION B: Weed Seed Removal Rates

EAST SITE

Contrast analysis of caged platter types during Year 1 indicated a strong difference (F=13.11, p<0.001) in overall percent loss between ARTH and TOTAL seed stations. However, there were instances when seed loss from the theoretical control platters (TOTAL) was more than that from the platters allowing arthropod access (ARTH). Averages with a negative number indicate such occasions. Sample size increased between years at the East site, from 24 sets of feeding stations in 2007 to 48 sets in 2008 and 2009. Methodology also differed slightly between years (TBL 2.1). Thus, results are interpreted on a per year basis.

Year 1 (2007)

Seasonal average of pigweed removal was 9.9% per week and there was no effect of spring tillage, insecticide use, or location within the field (block effect). Hairy nightshade was the seed most often removed from the free-choice platters in 2007, and mean removal was 11.7 percent per week, averaged across time and treatment. Contrast analysis indicated that neither spring tillage nor insecticide effects on removal of redroot pigweed varied over time (by week) in year 1.

Hairy nightshade seed removal was ten percent greater from strip-tilled plots than from conventionally tilled plots (18.1 vs. 7.9 percent per week) (F_{SOLSA}=10.79, p=0.008), when averaged across the year 1 season. ANOVA indicated that results were not consistent between the 6 blocks for this species (F_{BLOCK}=12.16, p=0.0005). Removal of hairy nightshade averaged 27.3% in the two blocks at the West end of the site location, 11.3% at the East end, and only 0.5% for blocks III and IV which were located in the middle of the field. Interestingly, only hairy nightshade seed loss varied between weeks. That is, during week 3
(23-30 August), ANOVA indicated an effect of spring tillage on removal rate of hairy nightshade ($F_{\text{SOLSA}} = 4.10$) but not wild-proso millet and Powell’s amaranth ($F_{\text{PANMI}}=0.17$; $F_{\text{AMAPO}}=0.42$) even though platters contained the three species offered at the same time.

There was an effect of insecticide on seasonal average removal of wild-proso millet ($F=3.94$, $p=0.075$). Seed loss of wild-proso millet was greater from untreated plots (11%/week) versus plots that had been sprayed with a broad-spectrum insecticide (3%/week). Blocking had no effect on this species, and contrast analyses indicated that variance of main effects between weeks was insignificant. Peak seed removal for all weed seed species occurred during 16-23 August 2007. During that time, the maximum percent seed loss per week was 23% for pigweed, 21% for hairy nightshade, and 14% for wild-proso millet.

**Year 2 (2008)**

Seed removal was evaluated during the assay year at the East site. Removal peaked during the week of 11 September 2008, and there were significant effects of both spring tillage ($F_{1,24}=6.15$, $p=0.06$) and insecticide ($F_{1,24}=5.70$, $p=0.06$) during that sampling period. However, there was no SPR*INS interaction revealed by ANOVA ($F_{1,24}=0.01$, $p=0.15$) for that week, nor any of the four other sampling periods in Year 2 (TBL 2.2). During the week of 11 September, average seed loss was 20.5% in areas that had been strip-tilled (ST) the previous season, versus 8.1% from conventionally - tilled (CT) plots. Additionally, loss averaged 20.7% per week in areas that had been sprayed with insecticide in 2007 (INS+), and only 7.8% per week in untreated areas (FIG 5). When seed stations were reset and evaluated one week later (26 September), average removal was 3.6% per week and there were no effects of either spring tillage ($F_{1,24}=0.01$, $p=0.93$) or insecticide use ($F_{1,24}=1.66$, $p=0.25$). These results suggest that seed loss is temporally variable, and may be influenced by previous disturbance.
Figure 5 – Effects of year 1 disturbance on seed removal of *A. powellii* during the week of 11 September 2008 (year 2). CT = Conventional spring tillage, ST = strip-till (reduced tillage). $F_{SPR, 1,24} = 6.15, p = 0.06, F_{INS, 1,24} = 5.70, p = 0.06$. Bars represent SEM±0.04.
Year 3 (2009)

During the final year at the East site, seed stations were set either with a single seed species (no-choice) or with the three species together (free-choice) (TBL 2.1). There was no significance of treatment effects on removal of seed species when offered alone. However, when seed platters contained all three species, and were presented late in the season (2-9 September), differences in removal rates were evident. Hairy nightshade removal, for instance, was influenced by spring tillage level in 2009 (Year 3) and averaged 5.5 % per day in conventionally-tilled plots and 1.6 % per day in reduced tillage plots (F=4.66, p=0.047). Similarly, pigweed removal was greatest in insecticide treated plots (4.3 % per day) versus untreated plots (1.9 % per day) during the final feeding trial. There were no treatment effects on removal of wild-proso millet in 2009, either when it was offered alone or paired with the other two seed species.

DISCUSSION

Activity-density of Carabidae and removal of weed seeds were temporally variable and differed by site. For the most part, tillage and insecticide applications imposed during the first year of a cropping rotation did not affect ground beetle AD for more than one year. However, when treatments were re-applied, existing patterns became more evident, suggesting that disturbance events have a cumulative effect on carabid AD. A similar Australian study found this same type of trend; that level of disturbance (tillage) was moderately significant in the first year, but that if treatments were repeated, the relationship between inputs and carabid AD was more evident (F=93.3, p ≤0.001) (Nash 2008). Early in the season, when carabid AD was steadily declining in most treatments, there was a slight increase in areas receiving reduced primary tillage and no insecticide the year before. This
could mean that areas of reduced disturbance are important havens for carabids during the early part of the season. Although carabid AD did not show any obvious patterns in year 2 at the East site, there was an effect of year 1 broad-spectrum insecticide use on the pattern of year 2 seed removal (FIG. 5). Overall, there was greater loss from areas that had been sprayed with broad-spectrum insecticides the previous season than from untreated areas (10.4 vs. 6.2 percent loss per week). The effect of insecticide was especially evident during the week of 11 September 2008 ($F_{1,34} = 5.70$, $p=0.06$), when removal of pigweed seed from insecticide-treated plots was 13% more than from untreated plots. Interestingly, this timing did not coincide with peak activity of beetles in Year 2. Rather, carabid AD was declining during that week (FIG.3, CE9) and was relatively similar in all treatments (between 0.3 and 0.5 BPN).

Bifenthrin, a pyrethroid commonly used in orchards and row crops, has a stated half-life in soil greater than 300 days (Fecko 1999). It was applied on 11 July 2007 and residual effects of the insecticide possibly were limiting the amount of invertebrate prey into the 2008 sampling season. A post-spray reduction in available prey could cause enhanced searching behavior, and thus increased activity of generalist predators (Chiverton 1984, Nash 2008). Another possibility is that population differences caused by imposed disturbances in year 1 resulted in a time-delayed and/or possible legacy effect on seed removal in year 2. We know of no other studies that report a difference in weed seed removal one full year after application of a broad-spectrum insecticide.

Carabid sampling in year 3 showed interesting temporal trends of treatment effects. In collection events during the early part of the season (April-May), the main effect of insecticide use was evident. Similar to year 1, AD was higher in insecticide-treated plots.
However, the first two collection events were performed before the re-application of insecticide (10 June 2009). Puzzlingly, in the weeks immediately following the application, there were no apparent effects, but significance of insecticide use appeared later in the season. This suggests that activity-density, as measured by pitfall trapping, is influenced by the use of a broad-spectrum insecticide, and that the significance of that effect varies throughout the season.

One of the most debatable aspects of ground beetle studies is the relationship between the density of crop vegetation and carabid abundance. Many claim that refuge strips interspersed throughout the crop (Lee 2001) or boundary areas surrounding the field (a.k.a hedge rows) (Dennis 1994) are important structures for the conservation of carabid populations. However, an important point was raised by Corbett and Plant (in Carmona 1999), when they challenged the fact that vegetative areas may serve as both a biological source and sink of carabid activity. That is, densely planted field edges or areas of low management serve as physical protection for carabids and make ideal egg-laying sites (Fadl 1996), thus perpetuating the continuation of a stable carabid community. Some species, in particular, are known to overwinter in areas of little to no disturbance (Dennis 1994, Fadl 1996). Thus, undisturbed areas may be a perpetual ‘source’ of beneficial insects.

Yet, it is also possible that field edges and untilled areas act as a biological ‘sink’, due to the increase of available prey common in unmanaged areas. An abundance of both floral and faunal prey might sustain carabids that would not otherwise reside in field boundaries or refuge strips. Artificial attraction to these areas could result in limited efficacy of predation out in the crop, where it is intended to occur. Granivorous species in particular, are reported to have greater diversity and abundance in neighboring refuge strips (Menalled 2007,
Lundgren 2006), which could have consequences for weed management potential within the cash crop. In our study, plastic fencing was installed to reduce the potential for movement between treatments, and the effects of tillage on ground beetle activity-density were less than expected. Nor did we see any effect of leaf canopy density on activity-density or seed removal. Removal of weed seeds did tend to be greater in areas of reduced tillage (ST) during certain time periods of our study, but it was not directly correlated with carabid activity-density, as measured by repeated pitfall sampling.
Chapter 3 - Assessment of the Relationship Between Seed Burial Depth and Weed Recruitment in Vegetable Cropping Systems of the Pacific Northwest

ABSTRACT

Integrated weed management involves combining chemical, cultural, and biological control tactics to help control the spread or persistence of targeted species. Cultural control tactics, i.e. reducing the intensity or changing the timing of tillage events, may help suppress certain species of summer annual weeds (Mohler and Galford 1997, Gallandt 2006, Peachey et al. 2004). No-till areas also tend to leave weed seeds on the soil surface, where they may be more susceptible to biological control via predation by rodents, birds, and granivorous arthropods (Navntoft 2006, Westerman 2003). In this study, we examined the impact of agricultural practices and seed burial depth on successful recruitment of weed seeds in two different field sites. Seed rows of three common weed species, Powell’s amaranth (Amaranthus powellii), hairy nightshade (Solanum sarrachoides), and wild-proso millet (Panicum miliaceum), were either sown at a depth of 2.5cm or placed directly on top of the soil. Planted seed rows remained undisturbed throughout the winter, and emergence of seedlings was measured the following summer (year 2) after no-till planting of squash (East site) or direct-seeding of corn (West site). Results varied between seed species and site location. At the East site, seed burial depth in year 1 was a significant factor on emergence in year 2 for both Powell’s amaranth ($F_{1,163}=94.59$) and wild-proso millet ($F_{1,163}=7.03$). Both species had greater emergence when seeds were sown directly on the soil surface. Effects of burial depth on recruitment at the West site were not consistent, and may have been influenced by the presence of an abundant pre-existing seedbank at that location. There was a significant interaction of spring tillage and insecticide use on recruitment of surface-sown seeds at both sites. Specifically, recruitment was greatest when weed seeds were left on the
soil surface and when plots were treated with broad-spectrum insecticides the previous season. This suggests that in addition to local effects such as seed placement and burial depth, recruitment is influenced by larger-scale agronomic practices.

KEYWORDS: Agro-ecology, weed recruitment, seed burial, conservation tillage


**INTRODUCTION**

Integrated pest management involves combining chemical, cultural, and biological control tactics to help control the spread or persistence of targeted species (Pedigo and Rice 2006). In the context of weed management, two methods of cultural control are to reduce the intensity or alter the timing of tillage. Tillage has the potential to have a major impact on weed seed survival and ultimately weed emergence (Mohler and Galford 1997). Tillage inverts the soil column which causes weed seeds to vertically shift (Benvenuti 2001), and recruitment of certain species is reduced if seeds are buried > 6cm below the surface (Peachey and Mallory-Smith 2007). Presumably, seeds remain buried indefinitely unless they are removed from the seedbank via other measures (Thompson 1987). Swanton and Booth (2004) review this concept by citing four major ways that weed seeds are removed from the soil, and how those processes may be manipulated in a weed management plan. One of the strategies is to increase seed mortality. When weed seeds are buried deep in the soil, they are more prone to microbial degradation and disease (Davis 2006, Sylvia 2005).

On the other hand, if soil disturbance is less intense (reduced-tillage), or eliminated completely (no-till, NT), weed seeds will be exposed to different mortality factors. For instance, when seeds are left on the soil surface, UV degradation and wind desiccation will
be greater. The risk of predation by granivores also increases for seeds that remain on the soil surface (Harper 1977, Thompson 1987). Rodents (Westerman 2003), birds (Navntoft 2009) and insects (Lundgren 2009) consume weed seeds and it is assumed that access to seeds is much greater if seeds remain on the soil surface, as is common in no-till systems. Additionally, NT areas may provide refuge and habitat for granivores such as carabid beetles (Gallandt 2006, Westerman 2003).

Because there are so many forces acting on weed seeds, factors that affect recruitment in different tillage systems are hard to distinguish experimentally. Therefore, a method of quantifying the impact of tillage on weed populations is necessary. For example, if no attempt is made to separate the effects of tillage from predation by arthropods from seed position in the soil, it may be impossible to determine which factor associated with a particular agricultural practice was responsible for the change in weed emergence patterns. In this study we assessed the effects of tillage, insecticides, and burial depth on emergence of three summer annual weeds common in the Pacific Northwest.

Pigweed (*Amaranthus* spp.) is considered bothersome due in large part to its annual seed shed (producing over 1700 seeds/m²) and extremely competitive nature with many crops (Forcella 1996). Another concern is the evidence of pigweed’s resistance to ALS-inhibitor herbicides. In fact, six different biotypes of Powell’s amaranth (*A. powellii*) showed significant resistance to imazethapyr in a recent study of Ontario soybeans (Ferguson 2001). Secondly, we focused on hairy nightshade (*Solanum sarrachoides*). This species is a large problem in row-crops in the Willamette Valley, particularly snap-beans, because of the highly-toxic berries it produces, which are difficult to avoid with harvesting equipment. Our third species of interest was wild-proso millet (*Panicum miliaceum*), a persistent weed that
has been present in North America for over 100 years. Wild-proso millet has a strong, pigmented seed coat that resists imbibition (Khan 1996) and results in the formation of a moderately persistent seedbank (Forcella 1996). Wild-proso millet forms a crop-weed complex with grain millet and until the recent registration of HPPD herbicides, was very hard to control.

By sowing seeds at two different depths in autumn, leaving them undisturbed throughout the winter, and measuring emergence the following summer, we sought to better discern which specific factors have the greatest impact on weed emergence and how agricultural practices might be combined to provide the best possible means of integrated weed control.

**MATERIALS & METHODS**

Research was conducted at two different locations on the OSU Vegetable Research Farm (Linn Co., OR). The first experimental site (EAST, 2007-2009) was a 2.5-acre field located on the eastern boundary of the research farm that had been in tall fescue rotation for more than 10 years prior. The second location (WEST, 2009-2010) was at the western edge of the farm and had a 10 year history of various vegetable and cover crop rotation. Soil at both sites has been classified as a Chehalis silt loam with 40:40:20 sand, silt, and clay, respectively. Each site was designed as a RCB factorial with 6 (East) or 5 (West) replications.

Experimental plots (10x20m) were defined in year 1 and exposed to one level of primary tillage in the spring. Conventional spring tillage (CT) was done by making repeated passes through the plot with a 45cm disc plough and a vertical tine roto-tiller. The other level of primary disturbance, reduced tillage, was done using a segmented rotary tiller, aligned to leave 0.45m strips of undisturbed vegetation between planting rows. This type of
tillage is commonly called strip-tillage (ST). Following primary tillage, an insecticide treatment was applied (INS + / INS -) to randomly selected plots. Bifenthrin (2-Methyl-3-phenylphenyl)methyl (1S,3S)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate; Discipline 2EC) is a broad-spectrum pyrethroid with a stated half-life of > 300 days (Fecko 1999). It was applied at a rate of 0.021 kg/ha using a CO₂ powered backpack sprayer equipped with a 305cm hand boom and 6-XR8003 nozzles set 51cm apart. An additional insecticide, Mocap 6EC™ ethoprop (1-(ethoxy-propylsulfanylphosphoryl) (Bayer Corp.), was sprayed either after (East site) or at the same time (West site) as the bifenthrin. Ethoprop was applied at a rate of 9.6L/ha using the same boom and nozzles as the bifenthrin application. Plots were irrigated post-spray to incorporate the insecticides.

Each field site was planted to snap bean (*Phaseolus vulgaris* var. Savannah) (Harris Moran, Modesto CA.) in the spring of year 1, at a rate of 20 seeds/m. There was a fall cover crop mixture (80:50 Steptoe barley (*Hordeum vulgaris*): common vetch (*Vicia sativa*) seeded at a rate of 24.4 kg/ha between growing seasons at each site. At the East location, plots were either conventionally-tilled, or not tilled before planting the cover crop. This treatment, referred to as fall tillage, effectively split main plots from 24 to 48 at the East site. Due to smaller plot sizes and apparent lack of significance at the East site, fall tillage was not an imposed factor at the West site; rather, all plots were direct seeded (24.4kg/ha) with barley and vetch in September of Year 1. Cover crop was sprayed down with glyphosate (2.2kg/ha) in the spring at both the East and West sites.

Weed seed rain was simulated after cover crop planting at each site by sowing three common weed species; Powell’s amaranth (*A. powellii*) (pigweed), hairy nightshade (*S.
sarrachoides), and wild-proso millet (P. miliaceum). Each planted strip contained 1000 pigweed, 250 hairy nightshade, and 500 wild-proso millet seeds, which were counted and sown by hand. Seeds were visually inspected for maturity and damage at time of processing. Weed strips, 1.8m long rows of mixed weed seeds, were placed either directly on the soil surface (SURFACE), or were planted 2.5cm below and covered with soil (BURIED). At the East site there were 2 strips of each burial depth at both ends of each subplot (n=192). At the West site, there were also 4 sown strips in each plot, but half of them (n=40) were covered with 1.5cm wire mesh screening at planting to reduce interference by rodents over the winter.

Year 2 vegetable crop at the East site was Cucurbita maxima var. Golden Delicious, which was direct-seeded at a rate of 2.7 seeds*m\(^{-1}\) using a cross-slot planter (John Deere, Inc.). Fertilizer (12-29-10 NPK) was banded next to the seed row at planting at 420 kg/ha. Squash was harvested on 16 October 2008. Sweet corn (Zea mays var. Coho) was planted in year 2 at the West site at a rate of 20 seeds/m. Fertilizer (16-16-16 NPK) was applied in row (477kg/ha) at time of corn planting.

Recruitment evaluation was conducted the following summer (year 2) at each site. On 28 Aug 2008, seedling emergence from the East site was evaluated by counting the number of visible stems of each species (Powell’s amaranth (pigweed), hairy nightshade, and wild-proso millet) within an approximate 15cm span of the planted strip. At the West site, wild-proso millet emergence was evaluated on 26 July 2010 by placing a measurement apparatus (1.8m long x 10cm wide) over planted weed-seed rows and all seedling stems within that area were counted. Pigweed and hairy nightshade recruitment was measured.
during the week of 28 July 2010 at the West site, after a period of heavy irrigation to encourage a flush of emergence of those two species.

Count data were square-root transformed (\(\sqrt{x+1}\)) before analysis, and analyzed using the PROC GLM function of SAS (SAS Institute, Inc., Cary, NC.), with error terms defined for a split-plot analysis. Seed strips sown on the surface were analyzed separately from those that were buried, rather than including all treatment effects in the same model, to retain sufficient degrees of freedom. Means were back-transformed to the original scale (number of seeds/1.8m) before reporting. Pearson correlation coefficients were used to compare data to other biotic observations taken during the experiment, such as percent weed cover and carabid beetle activity.

RESULTS

EAST SITE

Burial depth was a significant factor affecting the recruitment of two out of the three weed seed species. Therefore, each burial depth was examined separately for evidence of treatment effects (TBL 3.1). For both wild-proso millet (\textit{Panicum miliaceum}) and pigweed (\textit{Amaranthus powellii}), recruitment was greatest when seeds were sown directly on the soil surface the previous spring rather than buried 2.5cm (TBL 3.2). Mean recruitment of surface-sown pigweed was 5.9/1.8m strip versus 1.6 when seeds were buried below the surface (SEM ± 0.33, \(F_{1,163}=94.59\ p≤0.001\)) (TBL 3.2). Similarly, mean density of wild-proso millet was greatest when seed had been sown on the soil surface (7.0 seeds/strip) rather than at 2.5 cm below (4.9 seeds/strip) (SEM± 0.72, \(F_{1,163}=7.03, p≤0.01\)). There was, however, no effect of burial depth on hairy nightshade seedling recruitment at the East site (\(F_{1,163}=1.97, p=0.18\)).
This may be due to the low overall density of that species during the sampling date, averaging 0.4 stems/1.8m at date of evaluation. Based on initial seeding rates of 1000 pigweed, 500 wild-proso millet, and 250 hairy nightshade seeds for each strip (n=192), estimates of emergence of the three weed species were 0.37, 1.19, and 0.30 percent, respectively.

Burial depth seemed to affect weed seed recruitment at the East site. Additionally, there was evidence that large-scale, agronomic treatments influenced the emergence of some seed species. For instance, level of primary tillage (SPR) applied to the 240m² experimental plots in June 2007 may have influenced recruitment of surface-sown pigweed (A. powellii) in the spring of 2008. In conventionally tilled (CT) plots, mean emergence was 7.3 seeds per planted strip, versus 4.5 in reduced tillage plots (ST). The same effect was not evident for buried pigweed seeds (TBL. 3.2).

In addition to tillage, the broad-spectrum insecticide application in year 1 appeared to affect recruitment of Powell’s amaranth (pigweed) in year 2. Mean emergence from insecticide treated plots was up to 26% greater than emergence from untreated areas (F=7.07, p≤0.01). Although mean pigweed density was greater, for both surface and buried seeds, in insecticide-treated plots, the main effect of insecticide was only statistically significant for surface-sown seeds (TBL. 3.2).
Table 3.1 – ANOVA\(^a\) of main effects and interactions of year 1 treatments on recruitment of weed seeds in year 2 at the East site. Treatments were spring tillage (SPR), broad-spectrum insecticide use (INS), and fall tillage\(^b\) (FALL). Seeds were planted either a.) directly on the soil surface (SURFACE) or b.) 2.5cm below the soil surface (BURIED). AMAPO = *Amaranthus powelli*, pigweed; SOLSA = *Solanum sarrachoides*, hairy nightshade; and PANMI = *Panicum miliaceum*, wild-proso millet. Bolded values are significant at *p≤0.10, **p≤0.01, ***p≤0.001 (α=0.05).

### a.) Surface

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<th>p</th>
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<th>F</th>
<th>p</th>
<th>SOLSA</th>
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### b.) Buried

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<th>F</th>
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\(^a\) performed on square-root transformed (√(x+1)) seedling counts, evaluation date 28 August 2008.

\(^b\) weed seed rows were not disturbed during fall tillage; no variation was expected (or observed) for main effect of fall tillage.
Table 3.2 – Mean comparisons\(^a\) of year 1 (2007) plot treatment on recruitment of weed seeds in year 2 (2008) at the East site. Seeds were either placed on the soil surface (SURFACE) or buried 2.5cm (BURIED). Values in bold indicate a difference between means (ps0.1), alpha = 0.05. Spring tillage (Conventional tillage (CT) vs. strip-till (ST)), and broad-spectrum insecticide use (INS+ / INS-) were the main treatment effects applied to whole plots in year 1. Species codes are as follows: AMAPO = *Amaranthus powellii*, pigweed; SOLSA = *Solanum sarrachoides*, hairy nightshade; and PANMI = *Panicum miliaceum*, wild-proso millet.

<table>
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<th>PANMI</th>
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</table>

\(^a\) back-transformed(\(\sqrt{(x+1)^2}\)) means of seedling emergence 28 August 2008.
Figure 6 – Recruitment patterns\(^a\) of wild-proso millet (PANMI) and hairy nightshade (SOLSA) at the East site. Spring tillage (conventional (CT) / strip-till (ST)) and broad-spectrum insecticide (+/-) was applied in year 1 (2007). Results shown are from seeds that were sown on the soil surface in year 1; emergence was recorded in year 2. Bars = \(SE_{\text{PANMI}} \pm 0.25\), \(SE_{\text{SOLSA}} \pm 0.60\).

\(^a\)back-transformed means \((\sqrt{x+1})^2 - 1\) of seedling count data evaluated 28August 2008.
Granivorous arthropods, such as carabid beetles, often scavenge for weed seeds on the soil surface, and the evident relationships between insecticide use and seedling density add to the premise that the two may be related. For instance, effects of large scale plot management were evident for *A. powellii* seeds only if they had been placed directly on the soil surface. Pigweed is a small-seeded annual, with a very dense seed coat. A recent study from South Dakota reported that density and seed size affected removal preferences, and that *Harpalus pensylvanicus* showed a preference ($F_{1,7}=7.20$) for dense seeds such as common lambsquarters and redroot pigweed (Lundgren 2007). If this is the case and pigweed is a preferred species, it would help explain the difference in treatment effects between surface and buried seed that were apparent in our study. That is, if broad-spectrum insecticide use negatively affects surface-scavenging granivores, emergence of surface seed would be greater in areas where granivore populations were reduced. We saw this at the East site, where surface-sown seedling density averaged 13.37 per strip in plots treated with insecticide, versus 4.90 per strip from unsprayed areas ($F=7.07$, $p≤0.01$).

*A. powellii* was not the only seed species to show differences in recruitment depending on plot management. Hairy nightshade seed placed on the surface tended to have greater recruitment in insecticide treated (4.0 seedlings per strip) vs. untreated (0.27 per strip) areas. Additionally, for both surface-sown and buried seeds of *Solanum sarrachoides*, there was a significant interaction term between primary spring tillage and insecticide use ($F_{\text{SURFACE}} = 11.57$, $p<0.0001$, $F_{\text{BURIED}}=3.28$, $p=0.07$). When plots were conventionally-tilled in year 1 (CT), and not treated with insecticide (INS-), recruitment was greater than it was in strip-tilled (ST) plots. However, when broad-spectrum insecticides were applied the previous season, hairy nightshade recruitment was lower in conventionally-
tilled plots (FIG.6). Moreover, areas that were strip tilled and had insecticide applied (ST +), had the greatest amount of recruitment for surface-sown seeds. We saw this same pattern for wild-proso millet (FIG. 6), which suggests that the phenomenon is more than a mere consequence of hairy nightshade biology. For both species, emergence of seeds sown on the surface was greatest when plots had been strip-tilled and sprayed with insecticide the previous year.

WEST SITE

High natural densities of both hairy nightshade and pigweed may have obscured some of the treatment effects at the West site. Compared to the East field site, the plot chosen for experimentation in 2009-2010 had a relatively large pre-existing weed seedbank, due in part to the continuous vegetable cropping by previous researchers. However, wild-proso millet was not previously present at the West site, thus allowing a more precise evaluation of that species. Seed placement (i.e. burial depth) was a factor influencing recruitment of wild-proso millet but not the other two study species. Emergence of wild-proso millet at the West site was much greater for seeds that had been planted 2.5cm below the soil surface as opposed to directly on the soil surface. Each 1.8m strip of seeds had, on average, 67.4 seedlings from buried strips and 38.2 seedlings if seed had been sown on the surface ($F_{1,60}=12.5$, $p<0.001$).

Rodent exclusion provided by wire mesh cages over half of the sown strips ($n=40$) also had an effect on emergence of wild-proso millet ($F_{1,60}=2.57$, $p=0.11$). Emergence averaged 45.3 seeds per strip when rodents were permitted access and 58.3 when they were excluded. There was no interaction term for burial depth and mesh screening ($F_{1,60}=0.34$, $p=0.56$), suggesting that each factor was acting independently. Year 2 recruitment of wild--
Table 3.3 – Mean comparisons\(^a\) of year 1 (2009) plot treatment on recruitment of weed seeds in year 2 (2010) at the West site. Seeds were either placed on the soil surface (SURFACE) or buried 2.5cm (BURIED). Values in bold indicate a main effect difference between means (p≤0.1), alpha = 0.05. Spring tillage (Conventional tillage (CT) vs. strip-till (ST)), and broad-spectrum insecticide use (INS+ / INS-) were the main treatment effects applied to whole plots in year 1. Species codes are as follows: AMAPO = *Amaranthus powellii*, pigweed; SOLSA = *Solanum sarrachoides*, hairy nightshade; and PANMI = *Panicum miliaceum*, wild-proso millet.

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</tbody>
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\(^a\) back-transformed(\(\sqrt{(x+1)^2}\)) means of seedling emergence 26-29 July 2010.
proso millet was not affected by either spring tillage ($F_{1,60}=0.04, p=0.84$), or insecticide use ($F_{1,60}=0.06, p=0.80$) in year 1 (TBL 3.3).

Conversely, emergence of hairy nightshade at the West site did not seem to be influenced by either burial depth or rodent exclusion measures, but was affected by level of spring tillage performed in year 1. This effect was evident for both surface-sown and buried seeds. Mean seedlings per sown strip were 16.0 in conventionally-tilled (CT) plots and only 3.3 in reduced tillage (ST) plots, when burial depth was pooled (TBL 3.3). Failure to control hairy nightshade growth and seed production in year 1 was likely the cause of the apparent increase in CT plots in year 2.

DISCUSSION

Burial depth is one of the main factors affecting recruitment of summer annual weeds, and site-specific results for each studied weed species confirm that recruitment is a highly variable process. Each of the three species of interest (Powell’s amaranth, hairy nightshade, and wild-proso millet) has unique properties that likely influenced germination. For instance, pigweed seed, due to its small size and round shape would form a very persistent seedbank (Thompson 1987, Harper 1977). A low germination rate for buried $A. powellii$ seeds at the East site might support this theory. However, seed viability was not tested in our study, but Forcella (1996) reports that it can vary widely. In his study, $A. powellii$ (pigweed) seed viability was 11% in one year, and 59% the next.

A 2007 study of burial effects on hairy nightshade in the Pacific Northwest found that mortality and dormancy were higher for seeds buried near the surface versus seeds buried 6-25cm deep (Peachey and Mallory-Smith 2007). This supports the common notion that recruitment of $S. sarrachoides$ is often less in no-till versus conventionally-tilled systems. In
our study, emergence of hairy nightshade was relatively the same regardless of burial depth (0.4 vs. 0.2 seeds/strip at the East site and 10.2 vs. 9.6 seeds/strip at the West site).

However, as mentioned, evaluation of burial effects on this species was likely influenced by low recruitment and high noise at the East and West sites, respectively.

One interesting trend that was evident for hairy nightshade was an interaction between spring tillage and insecticide use in year 1 on the year 2 recruitment of surface-sown seeds (FIG. 6). Emergence of both species was affected by the spring tillage and broad-spectrum insecticide treatment of the previous year, regardless of seed position (TBL. 3.2). That is, for both surface and buried seeds, the effect of insecticide on recruitment of hairy nightshade and wild-proso millet was dependent on the intensity of spring tillage. Although means were very different between the two species, emergence patterns were nearly the same (FIG 6). In conventionally-tilled (CT) plots, the use of a broad-spectrum insecticide spray reduced emergence, but in strip-tilled (ST) areas, insecticide spray seemed to increase emergence. Notably, spring tillage level was markedly insignificant as a main effect on emergence of these two species (TBL. 3.1). This implies that it is the interaction of primary soil disturbance and broad-spectrum insecticide application that is influential, rather than the effect of either singular factor.

Seeds placed on the soil surface would presumably be more exposed to predation by granivores (Harper 1977, Thompson 1987) and we know from concurrent studies (Chapter 2) that removal of weed seeds was greatest in areas of reduced primary tillage, particularly when there had also been an application of broad-spectrum insecticides. Activity-density of carabid beetles is temporally variable and diet preferences may change throughout the season. It is possible that an increase in available prey items in unsprayed, conventionally-
tilled areas (CT - ), was great enough to satiate carabids enough that weed seeds were not consumed as voraciously as they might have been in plots treated with insecticide.

At the West site, emergence of buried seed was nearly 30% greater than emergence from surface-sown seed strips. The apparent difference between seed strips either covered or not covered by wire mesh suggests that predation by rodents was a factor influencing recruitment of wild-proso millet at the West site. Because wild-proso millet is a large-seeded species, it is likely a preferred food source of field mice and voles. Often, granivorous rodents depend on seed species that are readily available throughout the year (Hulme 1998, Harper 1977). The dark pigmented seed coat of certain wild-proso millet biotypes has been shown to guard against water uptake and imbibition (Khan 1996), and forms a moderately persistent seed bank. These qualities indicate that wild-proso millet likely offers an abundant and available source of nutrition for seed scavengers year-round.
Chapter 4 - Synthesis and Conclusion

“No other human occupation opens so wide a field for the profitable and agreeable combination of labor with cultivated thought as agriculture. I know nothing so pleasant to the mind as the discovery of anything that is at once new and valuable – nothing that so lightens and sweetens toil as the hopeful pursuit of such discovery. And how vast and varied a field is agriculture for such discovery.” - ABRAHAM LINCOLN, 1859

The time is ripe for a change in American agriculture. More aptly, it may be time to re-embrace the land management principles that were common in this country from 1830-1955. During that time, there was a national passion for land stewardship. With the installation of the U.S. Soil Conservation Service in 1935, and the fiscal support that followed, farmers began incorporating practices such as strip-cropping, direct-seeding, and planting around waterways. Just 20 years after beginning the soil conservation movement, there was a surplus of agricultural provisions (Little 1987). Imagine how welcome a problem that would be today, considering the global food and economic crises. As current demand for sustainable agriculture increases, so must our knowledge of how farm management affects beneficial insects and how habitats might be structured to reduce weed populations.

We have shown that disturbances such as tillage and broad-spectrum insecticides applied in the first year of a cropping rotation affected the activity-density (AD) of carabid beetles, but that effects were not evident in the assay year (year 2). One important exception to this is that seed removal rates during mid-September 2008 seemed to be influenced by plot management in 2007 (FIG. 5), where removal averaged 20% per week in plots that had either been strip-tilled (ST) or treated with insecticides (INS+). When plots were re-treated in a third year, AD and seed loss trends mirrored what was seen in first year data, suggesting a cumulative effect of plot management. Surprisingly, we saw no effect of
fall tillage on carabid beetle AD, seed loss, or weed seedling emergence at either site. At the East site, recruitment was greatest when seeds were sown on the soil surface, which implies that eliminating tillage may require growers to increase herbicide use for successful control. However, for two weed species, recruitment was high in plots with reduced tillage and insecticide (ST+), but lowest in ST- plots (Fig. 6). This suggests that spraying insecticide in strip-tilled areas could be detrimental to granivorous carabids residing there, and that the increase in weed recruitment may be a consequence of that effect.

There is still much work to be done to determine how effective carabids may be as biological weed control agents. How many seeds they consume per given area, for instance, needs to be quantified in order to make an estimate about how populations could contribute to weed control. While we did measure seed removal rates, we cannot be sure that removal was due to carabids alone, or that removal of seed equals predation. Behavioral diet studies are few and far between (Barney 1986, Saska 2004), but preliminary research shows that *P. melanarius* caches rather than immediately consumes large seeds such as wild-proso millet (N. Marshall, unpubl.; J. Pitcher, unpubl.), which could be important for management.

Planting depth of vegetable seed usually exceeds the known foraging capacity of carabid beetles (Thompson 1987). However, we know of no papers that address this question directly, especially for small, shallow-seeded crop seeds. We conducted a preliminary greenhouse study with both weed and crop seeds to examine the effects of seed burial depth on searching capacity of *P. melanarius*. Beetles were confined in 20L plastic totes and allowed to scavenge for planted seeds for approx. 1 week. We protected half of the seed rows with mesh screening, similar to the rodent-exclusion methodology in Chapter 3 at the West site. Within each bin, seeds were either buried at recommended depth
(Vesey™ Planting Guide) or placed on the surface. Each bin was watered by hand for 1 week and subsequent emergence was evaluated. We found that when radish and mustard seeds were placed on the surface and covered by exclusion screen, recruitment was much greater (84% and 95%, respectively) than when it was placed on the surface and left uncovered. These results suggest that carabid beetles would consume a small-seeded crop seed, if it were present on the soil surface. However, when radish seeds were buried ¼”, there was only a 5% difference between emergence of seeds that were exposed to carabids (no screen) and those that were protected. Based on these preliminary data, planting crops at recommended depths reduces the likelihood that carabid beetles will consume crop seeds. Also, due to sheer proportion of weed seeds to crop seeds usually present in a field, we feel confident that opportunistic carabids would be more likely to consume weed seeds.

Structuring crop habitat by incorporating conservation tillage may be beneficial for carabid beetles. The effect of broad-spectrum insecticides on Carabidae, however, deserves further review. Increased activity following an insecticide spray is probably a consequence of a reduction in prey availability, but it is also possible that beetles are able to escape toxicity, via spatial and/or temporal avoidance. Another idea worth investigating is that carabids inhabiting arable land have built up resistance to common insecticides, which may help explain why farmland is so dominated by just a few species. In our study, seed removal was often greater in insecticide-treated areas, which could reflect a shift in resource utilization by generalist predators in the absence of invertebrate prey. This could, in turn, impact the weed seed predation potential of carabid beetles. Nature is full of trophic relationships, and future studies should be mindful of these interactions so that we can continue to develop unique, effective, and sustainable pest management plans for agroecosystems.
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