TILLAGE AND COVER CROP EFFECTS ON WEED SEED FATE AND SOIL MICROBIAL ACTIVITY IN VEGETABLE CROPPING SYSTEMS

By

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ABSTRACT

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Strip-tillage (ST) and cover crops can protect and improve soils in vegetable production systems but their effects on weed communities and weed management are often poorly understood. The objectives of this study were to 1) determine the effects of tillage and cover crops on weed seed persistence and emergence, 2) evaluate the extent to which these effects were mediated by fungal pathogens, changes in herbicide efficacy, and light or oxygen exposure, and 3) investigate potential relationships between seed persistence and indicators of microbial activity. Seed persistence and emergence of Powell amaranth and large crabgrass were evaluated in long-term field trials with tillage (ST vs. full-width tillage [FWT]) and cover crop (no cover, rye, and vetch) treatments. Persistence of both species was higher under ST compared to FWT and could largely be explained by lower light exposure in ST. Rye cover cropping resulted in greater persistence of crabgrass seeds, but this could not be explained by fungal pathogens. Soil enzyme indicators of microbial activity including β-glucosidase, leucine aminopeptidase, and acid phosphatase were often higher under ST and rye cover crops and positively correlated with seed persistence. Results suggest that tillage and cover crop effects on emergence are variable due to complex interactions between herbicides, soil moisture, and nitrogen dynamics, all of which impact germination.

This thesis is dedicated
to my parents-I am who I am today because of you,
to my siblings- you have always been there for me,
to my friends- you have celebrated my quirks.
You are all my family; I am thankful.

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KEY TO ABBREVIATIONS

ai	Active ingredient
AMAPO	*Amaranthus powellii*
BG	β-glucosidase
CHEAL	*Chenopodium album*
DAP	Days after planting
DIGSA	*Digitaria sanguinalis*
EEA	Extracellular enzyme activity
FWT	Full-width tillage
GWC	Gravimetric water content
H_2O_2	Hydrogen peroxide
KCl	Potassium chloride
LAP	Leucine aminopeptidase
L-DOPA	3,4-dihydroxy-L-phenylalanine
MC	7-amino-4-methylcoumarin
MOLVE	*Mollugo verticillata*
MUB	4-methylumelliferone
NaOH	Sodium hydroxide
NH_4-N	Ammonium-nitrogen
NO_3-N	Nitrate-nitrogen
NT	No-tillage

PER	Peroxidase
PHEN	Phenol oxidase
PHOS	Acid phosphatase
PLFA	Phospholipid fatty acid
SOM	Soil organic matter
ST	Strip-tillage
SWMREC	Southwest Michigan Research and Extension Center
TZ	2,3,5-triphenyl tetrazolium chloride

CHAPTER ONE: Introductory Literature Review

Tillage and Cover Cropping. Agricultural practices aimed at conservation, including reduced tillage and cover crop use, provide many potential benefits in vegetable production systems. Conservation tillage includes a scale of operations ranging from no-tillage (NT) to reduced tillage (such as shallow or infrequent tillage). Another form of reduced tillage, referred to as strip-tillage (ST), focuses tillage in the crop row and leaves the areas between crop rows undisturbed. Compared to conventional tillage methods, soils under no-tillage have been shown to have improved soil quality at the surface (Runion et al. 2004) as well as nutrient stratification with greater availability at the surface (Hendrix et al. 1986; Lupwayi et al. 2006). In some cases strip tillage has an advantage over complete NT since it allows for the benefits of tillage in the crop row (including soil warming, fine seed bed preparation, incorporation of residue, and incorporation of fertilizers) while also gaining the benefits of NT in the rest of the field (including reduced soil organic matter loss, reduced soil erosion, moisture retention, and aggregate stability) (Brainard et al. 2013; Luna et al. 2012; Mochizuki et al. 2007).

However, the perceived and actual difficulties of weed management under these conservation tillage practices is one factor that has limited the adoption within vegetable production systems (Hoyt et al. 1994; Luna et al. 2012). While many studies have investigated NT systems (Leavitt et al. 2011), there is relatively little information regarding the long-term impact of ST on the population dynamics of important weed species, especially in vegetable crops. Many growers rely on full width tillage (FWT) methods (such as moldboard plowing and disking) to eliminate weeds from a field prior to planting (Brainard et al. 2013). Furthermore, if

cover crops are used in conjunction with NT or ST, cover crop residue remains on the surface and may make mechanical cultivation of weeds difficult. In a long term trial at the Southwest Michigan Research and Extension Center (SWMREC), previous data (Brainard and Frost, unpublished) suggest that ST resulted in an increase of large crabgrass (*Digitaria sanguinalis*) seeds and a reduction in broadleaf weed seed prevalence in the weed seedbank. Strip-tillage also resulted in higher weed seed-bank densities at shallower depths compared to conventional tillage and increased the persistence of Powell amaranth (*Amaranthus powellii*) seeds. However, the mechanisms responsible for changes in seedbank density are still unclear.

Cover crops have been proposed as a useful tool with benefits including improved soil fertility, soil moisture retention, improved soil tilth, and erosion prevention (Blevins et al. 1990; Kuo and Jellum 2002; Ranells and Wagger 1996; Teasdale 1996; Teasdale and Mohler 1993). Cover crops have also been shown to suppress weeds (Charles et al. 2006; Gallandt et al. 1999; Mohler and Teasdale 1993; Ngouajio et al. 2003; Williams et al. 1998), especially in reduced tillage systems. These plants can suppress weeds through a variety of mechanisms that prevent weed germination or growth (Price and Norsworthy 2013). Cover crops can be “smother crops” that shade-out or out-compete weeds for nutrients, water, light, and space. Some species of cover crop also release allelochemicals that can reduce weed seedling germination and growth (Barnes and Putnam 1983; Einhellig and Leather 1988; Weston 1996). In addition, both living cover crops and cover crop residue can provide physical barriers that prevent both the germination and growth of annual weeds (Crutchfield et al. 1986; Facelli and Pickett 1991).

However, cover crops can negatively impact weed management if not utilized properly. If a cover crop does not yield enough residue to act as a mulch barrier, weeds that germinate

might be more difficult to manage because of that cover crop residue and may even require high-residue cultivation equipment (Mirsky et al. 2013). While not always a possible or desirable option for growers, herbicides can be used to help control weeds in a RT system. However, while it is widely accepted that factors such as organic matter content and soil type can influence herbicide efficacy (Blumhorst et al. 1990), it is also possible for surface residues to reduce herbicide efficacy by either preventing adequate seedling/herbicide contact or by binding to the herbicide and rendering it ineffective (Banks and Robinson 1986; Buhler 1992; Locke and Bryson 1997). Regardless of the mechanism, when cover crop residue inhibits effective weed management and weeds are able to set seed, this increased seed rain can build-up the seedbank. It has been shown that as little as 10% of Powell amaranth biomass persisting to seed set is enough to produce higher quantities of seeds than in the initial seedbank (Brainard et al. 2011).

Weed Community Shifts under Conservation Agriculture. The concept of a "seedbank" is used to understand that seeds exist in the soil and persist over time. These seeds usually enter the seedbank as seed rain when shed from plants maturing in the field, but they may also enter via dispersal from neighboring fields (Booth et al. 2010).

While understanding the weed pressure in a field for any given year is important for successful production that year, the seedbank is important because the seeds within it can ultimately affect weed pressure and profitability in subsequent years. For successful long-term weed management it is important to minimize seed rain. Also potentially important arte identification of management practices which promote decay and predation of the seedbank.

Identification of the mechanisms influencing seed losses should be helpful in identifying practices that promote losses, thereby reducing long-term weed management costs.

Previous studies have demonstrated that conservation agriculture practices result in weed community shifts (Clements et al. 1996; Davis et al. 2005). Under ST management the seedbank community has been seen to shift away from summer annual broadleaf species and towards large crabgrass with an overall increase in perennial species as compared to conventional tillage (Brainard, unpublished data). Davis et al. (2005) found that conventional tillage (moldboard plow) and NT systems had grass-dominated seedbanks while reduced input and organic systems had seedbanks dominated by common lambsquarters (*Chenopodium album*) and common chickweed (*Stellaria media*). However, seedbank data from conventional and NT systems was found to have little value for predicting above-ground weed biomass. Other data has shown a decrease in overall seedbank size and a reduction of common lambsquarters in NT compared to conventional tillage using moldboard plow (Clements et al. 1996). In terms of weed density, large crabgrass and carpetweed (*Mollugo verticillata*) have been found to have higher densities in NT compared to conventional systems while common lambsquarters had greater density under conventional tillage (Teasdale et al. 1991).

The underlying causes of weed community shifts under conservation agriculture are often not well understood. For annual species, these shifts may be due to either differences in seed inputs or differences in the persistence of seeds in the soil. If conservation agricultural systems have reduced weed control efficacy than conventional production systems, then seed rain from weed escapes will result in greater contributions to the seedbank. Far less is known about differences in seed losses from the seedbank in conventional versus conservation

agricultural systems. Seeds leave the seedbank in one of many ways including death, pathogen attack, fatal germination, and successful germination (emergence).

Tillage events encourage seed germination through several mechanisms such as light exposure, increased soil temperature, increased soil aeration, and increased nitrogen mineralization (Mohler 2001). Therefore it would be expected that weed seed germination and subsequent emergence would be reduced under ST management and this effect has been seen in various crops (Brainard and Noyes 2012; Hendrix et al. 2004; Wang and Ngouajio 2008). Other things equal, reduced germination in conservation agricultural systems implies greater persistence. However, since these systems may also influence seed losses through changes in decay and predation, the net effect on persistence is difficult to predict without mechanistic studies identifying sources of those losses.

Microbes. Summer annual weed seeds within the seedbank may be exposed to decay agents including a variety of bacteria and fungi that reduce persistence through seed death. Some seeds come into contact with microorganisms while still on the parent plant, resulting in seedborne microbes. Seedborne bacteria recovered from field-produced weed seeds have included *Acinetobacter* spp., *Bacillus* spp., *Erwinia* spp., *Pseudomonas* spp., and *Xanthomonas* spp. (Kremer 1987). Once seeds are shed onto the soil and become incorporated into the seedbank, seeds may become exposed to additional soilborne microbes such as fungi including *Rhizopus* spp., *Pythium* spp., *Alternaria* spp., and *Fusarium* spp. (Wagner and Mitschunas 2008). An intensive review of European studies indicates that saprophytic fungi in the soil are important in reducing the weed seedbank of many species (Wagner and Mitschunas 2008). Fungi in the

soil can exude toxins that ultimately damage or kill seeds by preventing germination, destroying seed coats, or promoting solute leakage from cells (Halloin 1986; Harman 1983). Seedborne and soilborne fungi have had additive effects on seed persistence, with both microbial sources causing greater seed loss than either source alone (Kiewnick 1964).

Effects of Management on Microbes. Not surprisingly, agricultural management practices such as tillage and cover cropping can alter the abundance and diversity of microbes in the soil (Drijber et al. 2000). While the impact of strip tillage on fungal communities has not been closely examined there have been multiple studies on no-tillage systems. Since the between-row zones of a strip-tilled field are left un-tilled, the responses of microbial communities to no-till may be similar to those in the between-tow zone of a strip-tilled field. According to a meta-analysis by Wardle (1995), there is compelling evidence that no-till systems have greater microbial biomass than conventional tillage systems. Recent studies across the United States are consistent with these findings that microbial abundance and/or activity are greater under no-till (Frey et al. 1999; Helgason et al. 2009; Runion et al. 2004) especially when the reduced tillage system is combined with cover crop use (Minoshima et al. 2007). However, few such studies have been conducted in strip-till systems. Differences in disturbance patterns and spatial heterogeneity within strip-till can alter population dynamics of weeds compared to no-till (Brainard et al. 2013) and similar differences may occur across microbial communities.

The mechanism behind increased microbial abundance in conservation agriculture systems may be the result of increased soil moisture, decreased soil temperature, or changes in soil organic matter (SOM). Despite original suggestions that fungal abundance in no-till systems

is inversely related to soil moisture (Hendrix et al. 1986), fungal biomass has been found to be positively related to soil moisture as influenced by no-tillage and conventional-tillage operations (Frey et al. 1999) in which no-till soils consistently had greater water content compared to conventional-till soils regardless of a climatic gradient. However, Chen et al. (2007) found that soil moisture did not have a major effect on fungal biomass while there was a crop species effect on microbial community composition. At soil temperatures of 25-30^{0}C maximum fungal growth was observed with greater tolerance of lower temperatures than higher temperatures (Pietikäinen et al. 2005). While these results occurred in a controlled setting and not in the field they could suggest that fungi have reduced seasonal growth under systems that allow for warmer soils. Therefore fungi may have a competitive advantage over bacteria in systems that retain surface residues and maintain cooler soil temperatures. In the event that beneficial bacteria prevent pathogenic fungi from attacking seeds, these results could further indicate that cooler soil temperatures (such as seen in no-till or strip-till systems) could reduce beneficial bacteria abundance while promoting pathogenic fungi abundance and thus decreased weed seed persistence.

Differences in fungal biomass and activity have also been observed across soil depth and can be influenced by tillage and soil type. Fungal biomass has been shown to be greater in no-till versus conventional tillage systems at a shallow depth (typically 0-5cm) but not as consistently at deeper depths (Frey et al. 1999; Helgason et al. 2009; Lupwayi et al. 2004). However, Spedding et al. (2004) on a sandy loam/loamy sand found no such shift in fungal:bacterial abundance and there was not a significant effect of either tillage (conventional moldboard plow, reduced tillage, or no-tillage) or corn crop residue on fungal PLFA alone.

Studies on the effects of tillage on fungal abundance and activity show mixed results indicating the importance of understanding the effects of conservation agriculture practices on fungi specifically for Michigan vegetable production systems.

Fungal community biomass typically fluctuates during the course of a year and the timing of tillage operations can be an important factor in what responses are seen. In a cotton cropping system utilizing either no-tillage or conventional tillage, microbial community differences were affected by tillage with significant effects in February and May following spring tillage but not in October following fall tillage (Feng et al. 2003). There is evidence that microbial biomass and activity increase as a result of tilling residue into the soil, although this effect is short-lived after tillage operations (Lee et al. 1996; Lynch and Panting 1980). Given this range of results it is important to investigate how cover cropping and strip-tillage specifically will influence microbial activity temporally.

Enzymes as Indicators of Microbial Activity. While many studies have used phospholipid fatty acid analysis (Chen et al. 2007; Feng et al. 2003), or fatty acid methyl esters analysis (Drijber et al. 2000) to analyze soil microbial communities, microplate techniques for measuring extracellular enzyme activities are high through-put analyses successfully used to measure the function of soil microbial communities. Multiple enzymes that are released to break down organic matter can be assessed including β-1,4-glucosidase (BG), acid phosphatase (PHOS), leucine aminopeptidase (LAP), phenol oxidase (PHEN), and peroxidase (PER) (Saiya-Cork et al. 2002; Sinsabaugh et al. 2008). BG degrades cellulose primarily by hydrolyzing cellobiose to glucose (Ljungdahl and Eriksson 1985); PHOS hydrolyzes phosphomonoesters thereby

releasing organic phosphate (Toor et al. 2003; Turner et al. 2002); and LAP hydrolyzes leucine from polypeptides and is considered to be an indicator of peptidase potential (Sinsabaugh and Foreman 2001; Stursova et al. 2006). Phenol oxidase and peroxidase are two classes of enzymes primarily responsible for degrading polyphenols such as lignin and tannin (Kirk and Farrell 1987). Phenol oxidases are specifically able to degrade phenolic groups (Mayer and Staples 2002) while peroxidases degrade aromatic compounds (Hofrichter 2002).

Links between Soil Microbial Activity and Seed Persistence. Although conservation agricultural systems tend to increase microbial biomass and diversity, it is unclear what impact those changes have on the persistence of weed seeds. Previous studies suggest that both fungi and bacteria colonize seeds (Kremer, 1987; Wagner and Mitschunas, 2008) and that saprophytic fungi play an important role in reducing seed persistence (Wagner and Mitschunas, 2008). If fungi are a primary driver of seed decay, then shifts in management toward fungal dominated communities may reduce persistence. In addition, Chee-Sanford et al. (2006) speculate that beneficial microbes may help to protect seeds by producing antimicrobial compounds that prevent against attacks from harmful microbes. However, few studies have examined potential links between crop management, microbial diversity, and the persistence of specific weed species. More detailed information on specific changes in microbial communities and their relationship to persistence of problematic weed species should be helpful in predicting and manipulating seed decay to reduce long-term weed management costs.

Summary. The growing interest for conservation agriculture and the practices of strip-tillage and cover cropping necessitates a better understanding of the effects these practices have on weed management. Although previous research has shown shifts in weed population dynamics under conservation agricultural systems, the mechanisms behind those shifts are often unclear. Nor is it clear whether weed seedbank dynamics in strip tillage systems differ from those in no-till systems due to their greater spatial heterogeneity. Specific areas of interest involve the spring emergence of summer annual weeds as well as the long-term seed persistence of those weeds. Tillage and cover crops effects on emergence and persistence are likely mediated by conditions specific to the type of tillage and cover crops used, as well as the cropping system in which they are embedded. Few previous studies have examined in detail the impacts of conservation agricultural systems on seedbank dynamics within vegetable cropping systems. Attention will be given here not only to the effects of strip-tillage and a winter rye cover crop on summer annual weed emergence and seed persistence but also to the explanatory factors of light, fungal pathogens, and microbial activity.

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CHAPTER TWO: Tillage and Cover Cropping Impacts on Weed Emergence- Herbicides and Fungal Pathogen Interactions

Abstract

Strip-tillage and cover crops can reduce input costs while protecting and improving soils. However weed management under strip-tillage can be challenging, especially for vegetable crops with limited herbicide options. The effects of tillage and cover cropping on weed emergence are highly variable and reflect both long-term changes in the weed seedbank as well as short term effects on seed germination and pre-emergence mortality. Objectives were to evaluate both the short and long-term effects of tillage (full width tillage [FWT] or strip tillage [ST]) and cover crops (none, winter rye or vetch) on weed emergence; and to evaluate the extent to which these effects were mediated by fungal pathogens or changes in herbicide efficacy. Tillage and cover crop treatments were imposed on the same plots for six years in a sweet corn-snap bean-cucurbit rotation in two adjacent fields on sandy soils in SW Michigan. In year 7 in each field (2015 and 2016), herbicide and fungicide sub-subplots were established. Herbicide treatments consisted of either no herbicide application, or an application of S-metolachlor one day after planting (DAP). Seeds of Powell Amaranth and large crabgrass that were either untreated or coated with fungicide (captan, trifloxystrobin and metalaxyl) were sown in separate sub-subplots not receiving herbicides. Emergence of ambient Powell amaranth and common lambsquarters was greater under ST compared with FWT and greater under rye cover crops as compared with no cover crop and vetch cover crop treatments. Emergence of sown seeds of Powell amaranth and large crabgrass was suppressed in one of two years in ST+rye compared to ST+vetch and the no cover control treatments. In one of two years

fungicide-treated Powell amaranth seeds had greater emergence compared with untreated seeds, but this effect was independent of tillage and cover crop treatment. The efficacy of S-metolachlor on common lambsquarters was reduced in ST+rye or ST+vetch compared with FWT and no cover crop treatments. These results suggest that 1) fungal pathogens did not play a role in the observed effects of tillage and cover crops on weed emergence, and 2) S-metolachlor efficacy was reduced by both vetch and rye cover crop surface residues.

Nomenclature. Powell amaranth, *Amaranthus powellii* S. Wats. AMAPO; large crabgrass, *Digitaria sanguinalis* (L.) Scop. DIGSA; common lambsquarters, *Chenopodium album L.* CHEAL; carpetweed, *Mollugo verticillata L.* MOLVE; winter rye, *Secale cereal*; hairy vetch, *Vicia villosa*.

Key Words. Strip-tillage, herbicide efficacy, S-metolachlor, fungicide, captan, trifloxystrobin, metalaxyl.

Introduction

The impact of weeds on crop yield and quality depends on weed emergence during crop establishment (Hoyt et al. 1994). The effects of tillage and cover cropping on weed emergence are highly variable and reflect both long-term changes in the weed seedbank as well as short-term effects on seed germination and pre-emergence mortality. Understanding the mechanisms for differences in weed emergence and exploiting this understanding to reduce emergence is a potentially helpful approach for reducing both yield loss and weed management costs, particularly in conservation agricultural systems where weed management is often a major constraint (Hoyt et al. 1994).

Tillage and Cover Crop Effects on Emergence. Conservation tillage and cover cropping can influence emergence of weeds by changing seed distribution within the soil profile (Cardina et al. 1991; Clements et al. 1996; Cousens and Moss 1990; Yenish et al. 1992), altering soil conditions affecting germination (Blevins et al. 1983; Dyer, 1995; Haramoto and Brainard 2017), and by reducing herbicide efficacy (Locke and Bryson 1997). Vertical distribution of seeds within the seedbank changes based on tillage (Clements et al. 1996). No-till systems are typically characterized by shallower distribution of weed seeds in the soil profile thereby increasing potential emergence of many summer annual weeds, other things equal. However, in the short-term, tillage also often stimulates emergence by promoting seed germination through its effects on soil temperature, moisture, oxygen, and nutrient status (Mohler 2001).

Cover crop residues influence weed emergence both when incorporated into the soil (Kumar et al. 2009; Radicetti et al. 2013) as well as when left on the soil surface (Bernstein et al. 2014; Davis 2010; Mirsky et al. 2011). Previous studies have demonstrated that tillage and cover crop effects on weed emergence are mediated in part by soil moisture, however, other factors including temperature, nitrogen availability, fungal pathogens, and allelopathic effects also often play a role (Haramoto and Brainard 2017; Kumar et al. 2009; Mohler et al. 2012; Weston 1996).

Role of Fungi in Mediating Emergence Responses. The potential role of microbes in mediating tillage and cover crop effects on weed emergence has been explored in several studies. Mohler et al. (2012) incorporated oats and pea cover crop residues and found a reduction in weed seedling emergence due to *Fusarium* spp. rather than allelopathic chemicals. Differences in emergence between cover crop and no cover crop controls largely vanished when soils were sterilized to eliminate soil fungal pathogens. In another study a fungal pathogen, *Pythium ultimum,* decreased the emergence of velvetleaf (*Abutilon theophrastii)* by increasing the fatal germination of seedlings (Davis and Renner 2007). The suppressive effect of buckwheat on emergence of shepherd's-purse (*Capsella bursa-pastoris*) and corn chamomile (*Anthemis arvensis*) was dependent upon seed treatment with fungicides, but other factors, including nitrogen and allelochemicals, were more important in explaining suppression of some species (Kumar et al. 2008). Together these studies suggest that soil-borne pathogenic fungi can have significant impacts on weed seedling emergence especially in the context of conservation management.

Herbicide Interactions with Tillage and Cover Crop Residue. It is widely accepted that factors such as soil organic matter (SOM) content and soil type can influence herbicide efficacy (Blumhorst et al. 1990). Across soil types, the herbicide metolachlor has increased biodegradation and greater sorption where there is greater soil organic matter of surface soils (Rice et al. 2002). This suggests S-metolachlor, an active isomer of metolachlor, may have reduced efficacy in soils with reduced tillage and cover cropping legacies which often have greater SOM. In addition, it is also possible for surface residues to reduce herbicide efficacy by either preventing adequate seedling/herbicide contact or by binding to the herbicide and rendering it ineffective (Banks and Robinson 1986; Buhler 1992; Locke and Bryson 1997). Burgos and Talbert (1996) and Teasdale (1993) reported reduced efficacy of atrazine plus metolachlor in the presence of hairy vetch residue and attributed this effect to bother interception of the herbicide and maintenance of higher soil moisture under cover crop residue compared to bare soil. This may not always be the case, however, especially at low cover crop and herbicide rates. Teasdale et al. (2005) found that when a hairy vetch cover crop and pre-emergence application of metolachlor were used at low rates, there was a synergistic effect on reducing weed emergence. However, these results have not been confirmed by other studies and relatively little is known about specific effects of cover crop residues (e.g. winter rye and hairy vetch) on S-metolachlor efficacy. Since S-metolachlor is a commonly used herbicide in many crops for which conservation practices are being investigated and adopted, improved understanding of its interactions with these practices is important for development of integrated weed management systems for conservation agriculture.

Objectives and Hypotheses. The primary objectives of our research were to 1) evaluate the interactive effects of tillage and cover crops on the emergence of summer annual weeds within a vegetable cropping system and 2) evaluate the role of fungal pathogens and herbicides in mediating these effects. We hypothesized that:

1) In the absence of herbicides, weed emergence would be lower in ST compared to FWT and lower in cover crop compared to no cover crop treatments.
2) In the presence of herbicides, weed emergence would be greater in ST treatments with cover crops because cover crop surface residue will act as a physical barrier between herbicides and the soil, thereby reducing herbicide efficacy.
3) The effect of fungicide seed treatment on weed emergence would be greatest in cover crop and ST treatments because fungal pathogens mediate tillage and cover crop effects on weed germination or pre-emergence mortality.

Materials and Methods

Long-term Trial Experimental Treatments and Design. Two summer annual weed emergence experiments (Experiment 1 and Experiment 2) were conducted twice within a subset of treatments in two long-term tillage trials located at the Southwest Michigan Research and Extension Center in Benton Harbor, Michigan (42.085244^0 N, 86.358736^0 W). The long-term trials were initiated in September 2008 and September 2009 on Oakville fine sand and followed a three year rotational sequence of sweet corn-snap bean-cucurbit crops (butternut squash in 2011 and 2012, pickling cucumber in 2014 and 2015) that were offset by one year. Experimental treatments for each field included all combinations of three factors: tillage (strip-tillage [ST] vs conventional full-width tillage [FWT]), cover crop (no cover crop, winter rye, or either a hairy vetch winter rye mix until 2014 and 2015 or hairy vetch since 2015 and 2016), and weed management (standard grower practice [high] vs. reduced input [low]). Treatments were imposed in the same plots each year. Plots were arranged in a split-split plot design with tillage as the main plot factor, cover crop as the sub-plot factor, and weed management as the sub-sub plot factor. Tillage main plots measured 11.4m x 18.3m and were arranged in a randomized complete block design with four replications. Weed management split-split plots were 3.8m x 9.1m with either two (winter squash) or five rows (all other crops) per plot. Weed emergence experiments were conducted only in the low weed management sub-sub plots of these long term experiments (Table 2.1) to avoid any confounding effects of historical weed management intensity on weed emergence.

Table 2.1: Trial design and treatments

Factor	Treatment	Levels
Sown Seed Emergence		
Whole plot	tillage	strip-tillage, full-width tillage
Sub plot	cover crop	no cover crop, winter rye, hairy vetch
Sub-sub plot	fungicide	triple-fungicide coating, no fungicide
Ambient Weed Emergence		
Whole plot	tillage	strip-tillage full-width tillage
Sub plot	cover crop	no cover crop, winter rye, hairy vetch
Sub-sub plot	herbicide	pre-emergence herbicide, no herbicide

Field Management. In early September of each year, winter rye and vetch cover crop treatments were drilled at 125kg/ha and 62kg/ha using a grain drill with 19cm between-row spacing (Table 2.2). Tillage occurred in May or early June depending on the crop. Full width tillage consisted of moldboard plowing followed by disking and field cultivating. Strip-tillage was accomplished using either a Hiniker 6000 strip-tiller (for sweet corn, snap beans, and cucumbers) or an Unverferth 120 subsoiler (for winter squash). Both strip-tillage implements were equipped with a row-cleaner (to remove cover crop residue), a shank, offset disks, and a rolling basket. Strip tillage resulted in an approximately 25cm wide by 30cm deep zone of disturbed soil into which crops were planted.

Weed management in the low intensity treatments varied by crop, and included both herbicides and mechanical cultivation. In snap beans and sweet corn, herbicides included S-metolachlor (Dual Magnum, 1 pint/acre) pre-emergence with a post-emergence application of

sodium salt of bentazon (Basagran, 0.75 quarts/acre) and fomesafen sodium salt (Reflex, 0.5 pint/acre). In some years, snap beans also received a post-emergence application of clethodim (SelectMax, 1 pint/acre) to control grass weeds as needed. For cucurbit crops, herbicides included a pre-emergence application of ethalfuralin/clomazone (Strategy, 3 pints/acre) and a post-emergence application of clethodim (SelectMax, 1 pint/acre). In FWT treatments, cultivation with s-tine sweeps was also used as needed to manage weed escapes between crop rows. Rates and timings of herbicide applications differed slightly by year and crop but were identical for all treatments within a given year.

Table 2.2: Timing of relevant field operations and experimental procedures.

Date	Field Operation	Experiment Operation
2014 Field One		
Jul-28	pickling cucumber harvested	
Sept-2	field disked, rye and vetch cover crops planted	
2015 Field One		
May-20	cover crops terminated	
Jun-2	conventional tillage event	
Jun-3	strip-tillage event	AMAPO+fungicide, DIGSA+fungicide, AMAPO, and DIGSA seeds sown
Jun-4	sweet corn planted	herbicide excluding plastic placed in field
Jun-5	Pre-emergence herbicide application	plastic removed after herbicide application
Jun-11		soil samples collected
Jun-16?		soil samples collected
Jun-22		emergence counts, soil samples collected
2015 Field Two		
Jul-22	pickling cucumber harvested	
Sept-10	field disked, rye and vetch cover crops planted	
2016 Field Two		
May-19	cover crops terminated	
Jun-1	conventional and strip-tillage events	

Table 2.2 (cont'd)

Jun-2	sweet corn planted	AMAPO+fungicide, DIGSA+fungicide, AMAPO, and DIGSA seeds sown, soil samples collected, herbicide excluding plastic placed in field
Jun-3	pre-emergence herbicide application	plastic removed after herbicide application
Jun-9		soil samples collected
Jun-10	solid-set irrigation installed and ran	
Jun-13	irrigation ran	
Jun-16		soil samples collected, temperature pendants buried
Jun-21		Emergence counts, soil samples collected
Jul-17		temperature pendants retrieved

Experiment 1: Sown Seed Emergence. *Experimental design.* This experiment evaluated the effects of tillage (ST vs. FWT), cover crop (no cover, rye, or vetch) and seed treatment (fungicide treated vs. untreated) on emergence of Powell amaranth (*Amaranthus powellii* S. Wats., AMAPO, collected in 2011 from Hickory Corners, MI) and large crabgrass (*Digitaria sanguinalis* (L.) Scop., DIGSA collected in 2012 from Benton Harbor, MI). Seeds were sown in all low weed management sub-sub plots of the long-term trials described above (Table 2.1). For each weed species the design was a split-split plot design with tillage as the main plot factor, cover crop as the sub-plot factor, and fungicide as the sub-sub plot factor.

Seed preparation. A subset of seeds from both species were coated with a triple fungicide treatment used in previous studies to protect weed seeds against fungi including *Rhizoctonia*, *Fusarium*, *Pythium*, and *Phytophthora* (Kumar et al. 2008, 2011). This coating contained captan (Captan Fungicide, 71 mg ai/100 g seed, Southern Agricultural Insecticides, Inc.), trifloxystrobin

(Flint, 10 mg ai/100 g seed), and metalaxyl (Apron 35 SD, 15 mg ai/100 g seed). Untreated seeds were from the same seed lot but received no fungicide coating.

In year seven for each field (2015 for field one and 2016 for field two), amaranth and crabgrass seeds were sown in a 0.25 m^2 quadrat within all low weed management plots within one day of tillage (Table 2.2). Seeds were sown by removing surface cover crop residue, planting seeds 0.6 cm below the soil surface, and replacing the cover crop residue. Seeds were sown within unsprayed micro-plots established by temporarily placing a 0.5 m^2 plastic sheet in the between-row zone during S-metolachlor application (Dual Magnum, 0.25L/hectare=1.3 pints/acre, Syngenta) one day after planting (DAP) sweet corn. In 2016, herbicide application included S-metolachlor at 0.19L/hectare= 1pint/acre with glyphosate (Roundup, 0.38L/hectare =1qt/acre, Monsanto).

Experiment 2: Ambient Weed Emergence. *Experimental design.* This experiment evaluated the effects of tillage (ST vs. FWT) and cover crops (no cover, winter rye, or vetch) on herbicide efficacy and ambient weed emergence. Natural weed emergence was monitored in all low weed management sub-sub plots of the long term trials as well as within the unsprayed micro-plots established in Experiment 1. For each weed species the design was a split-split plot with tillage as the main plot factor, cover crop as the sub-plot factor, and herbicide as the sub-sub plot factor.

Data Collection. Emerged seedlings of sown seeds were counted 19 days after planting (DAP). In addition, ambient summer annual weed emergence counts were taken 19 DAP. Counts were

taken from 0.25 m^2 quadrats within each non-herbicide treated micro-plot as well as from a 0.25 m^2 quadrat from a complementary herbicide treated area in the between-row zone of each plot. Species counted included Powell amaranth, large crabgrass, common lambsquarters (*Chenopodium album L.*, CHEAL), carpetweed (*Mollugo verticillata L.*, MOLVE), and ladysthumb (*Polygonum persicaria L.*, POLPE). Herbicide efficacy within each tillage x cover crop sub-plot was determined as:

$$(1)\ \%\ Control = ((E_{nh} - E_h)/E_{nh})*100$$

Where E_{nh} is emergence in the no herbicide sub-sub plot and E_h is emergence in the herbicide sub-sub plot.

Soil Sampling and Processing. Shallow soil samples were collected 8, 13, and 19 DAP in 2015 and 0, 7, 14, and 19 DAP in 2016. Samples were collected adjacent to sown seeds (AMAPO + fungicide, DIGSA + fungicide, AMAPO and DIGSA) in unsprayed micro-plots by removing cover crop residue, skimming soil from the surface to approximately 1.3 cm depth using a scoopula, and replacing the cover crop residue. Subsamples of the 2016 soil samples only were weighed fresh, dried at 100^{0}C for 48 hours and reweighed. Gravimetric water content (GWC) was then calculated according to:

$$(2)\ GWC = ((\text{weight of wet soil} - \text{weight of dry soil})/(\text{weight of dry soil}))*100$$

The remainder of all samples were dried at 40^{0}C, ground, and processed using a 1M KCl extraction technique modified from (Keeney and Nelson 1987). Extracts were frozen before being tested for nitrate-N using a cadmium reduction technique modified from the Griess-Ilosvay method and for ammonium-N using the ammonium-salicylate method. Samples were analyzed using a Lachat flow injection autoanalyzer (Hach Co., Loveland, CO).

Soil Temperature Monitoring. HOBO Onset temperature pendants (UA-001-08) monitored soil temperature in unsprayed micro-plots of all rye and no cover treatments in 2016. Sensors were placed at approximately 1.3 cm depth in the soil by removing surface residue, burying the pendant, and replacing the residue. Measurements were recorded every 30 mins starting 14 days after weed seeds were sown.

Statistical Analysis. Emergence for all species was defined as seedlings that had germinated and grown above the soil surface or cover crop residues. Proportion of emergence for each sown seed treatment was then calculated as the number of emerged seeds divided by the number of seeds initially buried. Ambient weed emergence was determined as the number of seedlings per unit of area. There were insufficient emergence counts of ladysthumb for meaningful statistical analysis and therefore it was excluded from analysis. All analyses were conducted separately for each year using Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC). The effects of tillage, cover crops and fungicide treatment on sown seed emergence were analyzed separately for each species using an analysis of variance (ANOVA) for each year. Analyses were conducted using the PROC GLIMMIX procedure with tillage,

cover crop, and fungicide treatment as fixed effects and rep, rep x tillage, and rep x tillage x cover crop as random effects. The effects of tillage and cover crops on soil moisture (2016 only) and soil ammonium and nitrate were analyzed using the PROC GLIMMIX procedure with tillage and cover crop as fixed effects and rep and rep x tillage as random effects. The effects of tillage and cover crops on herbicide efficacy and ambient weed emergence were analyzed using PROC GLIMMIX with tillage, cover crop, and herbicide as fixed effects and rep, rep x tillage, and rep x tillage x cover crop as random effects. Soil temperature readings were averaged for days (08:00-19:30) and nights (20:00-07:30) during the emergence period before completing PROC GLIMMIX analysis with tillage and cover crop as fixed effects and rep and rep x tillage as random effects. Where needed the data were transformed using log, square root, or squaring procedures to better meet normality assumptions. Treatment means separation occurred using Fisher's Protected LSD at α=0.05. Correlation analyses between soil inorganic nitrogen and ambient weed emergence, soil inorganic nitrogen and sown seed emergence, soil temperature and ambient weed emergence, and soil temperature and sown seed emergence were all conducted separately using the PROC CORR procedure of SAS.

Results and Discussion

Experiment 1: Sown Seed Emergence. *Effect of tillage and cover crops on emergence.* Cover crops and tillage individually were less important for influencing emergence and, instead, sown seed emergence was greatly impacted in treatments where winter rye residue remained on the surface, with lowest crabgrass and amaranth emergence in ST+rye plots in 2015 compared to all other treatments (Table 2.3; Figure 2.1). This is similar to other studies that saw an impact on weed emergence by cover crop surface residues (Bernstein et al. 2014; Davis 2010; Mirsky et al. 2011). In 2016 amaranth had lower emergence in ST plots compared to FWT, regardless of cover crop treatment, although this effect was only marginally significant (P=0.0948; Table 2.3). In contrast, crabgrass emergence was unaffected by tillage in 2016 and vetch reduced crabgrass compared to the no cover crop control, regardless of tillage. Crabgrass emergence in winter rye plots was not statistically different than either no cover crop or vetch treatments.

Table 2.3: Main effects, interactions, and overall ANOVA for sown seed emergence of amaranth and crabgrass for 2015 and 2016

	Powell Amaranth		Large Crabgrass	
	2015	2016	2015	2016
	number emerged / number sown			
Tillage Main Effect				
FWT	0.2440 a	0.2369 a	0.2834 a	-
ST	0.0676 b	0.1441 a	0.2200 b	-
Cover Crop Main Effect				
No cover crop	0.2232 a	-	0.3327 a	0.2293 a
Rye	0.0601 b	-	0.1481 b	0.1998 ab
Vetch	0.1710 a	-	0.2744 a	0.1238 b
Fungicide Main Effect				
With fungicide	0.1579 a	0.2232 a	-	-
Without fungicide	0.3567 a	0.1552 b	-	-
Tillage x Fungicide Interaction				
FWT				
With fungicide	-	0.2475 a	-	-

Table 2.3 (cont'd)				
Without fungicide	-	0.2266 a	-	-
ST				
With fungicide	-	0.2002 a	-	-
Without fungicide	-	0.0972 b	-	-
Tillage x Cover Crop Interaction				
FWT				
No cover crop	0.2982 a	-	0.3362 a	-
Rye	0.2035 abc	-	0.2634 a	-
Vetch	0.2350 ab	-	0.2506 a	-
ST				
No cover crop	0.1589 bc	-	0.3291 a	-
Rye	0.0016 d	-	0.0328 b	-
Vetch	0.1171 c	-	0.2981 a	-
ANOVA				
Tillage	**	+	*	N.S.
Cover Crop	***	N.S.	***	+
Fungicide	+	*	N.S.	N.S.
Tillage x Fungicide	N.S.	+	N.S.	N.S.
Tillage x Cover Crop	**	N.S.	***	N.S.
Cover Crop x Fungicide	N.S.	N.S.	N.S.	N.S.
Tillage x Cover Crop x Fungicide	N.S.	N.S.	N.S.	N.S.

*Where different letters indicate significant differences between treatments at α=0.05 and the above P-values are statistically significant at the following α: +<0.10, *<0.05, **<0.01, ***<0.001*

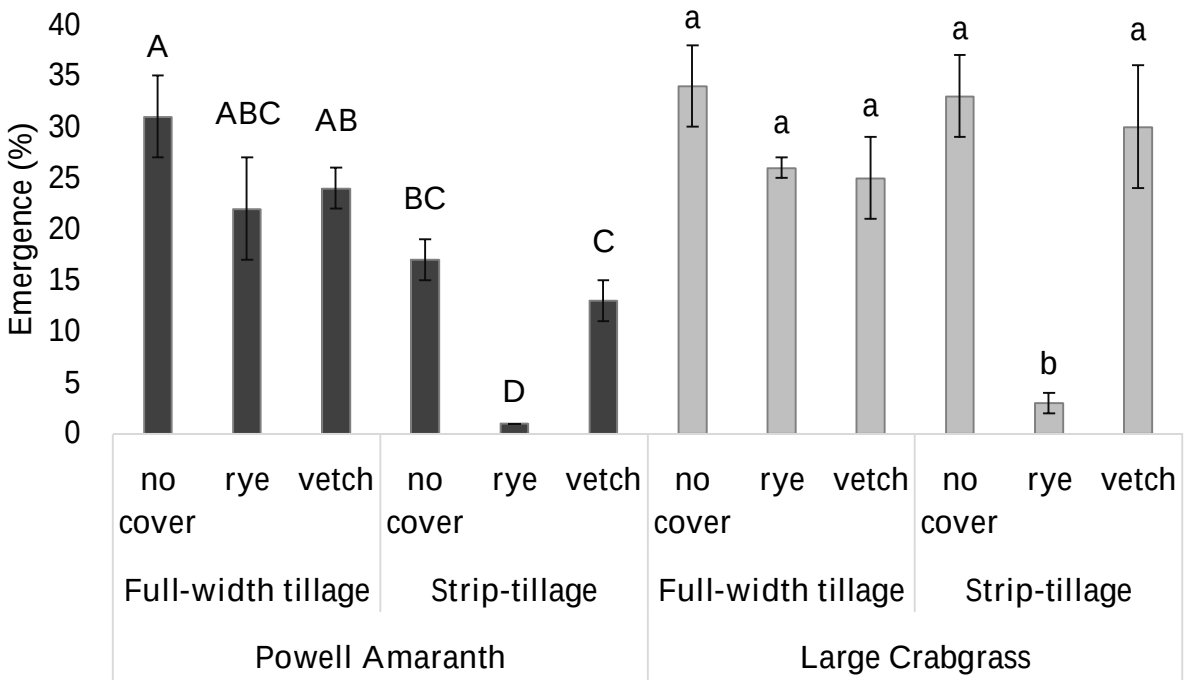

Figure 2.1: Tillage and cover crop effects on sown seed emergence in 2015. Different letters indicate significant differences between treatments within species at α=0.05.

Effect of fungicide on emergence. Large crabgrass emergence was not significantly influenced by fungicide treatment in either year of this study (Table 2.3). Fungicide coated Powell amaranth seed had greater emergence compared with non-coated seed in 2016, but not in 2015 (Table 2.3), suggesting that 1) treated seeds were protected from fungal pathogens that reduce weed emergence, or 2) the fungicide coating itself promoted germination of those seeds and increased emergence. In petri dishes, we found evidence of fungicide-treated amaranth seeds having greater germination but results were inconsistent over multiple trials. Large crabgrass seeds germinated in petri dishes did not show a fungicide effect.

The lack of an interaction between fungicide use and either tillage or cover crop treatments in either year (Table 2.3) indicates that fungal pathogens did not mediate tillage or cover crop effects on weed seed emergence. One possible exception to this was the marginally

significant (P=0.0919) interaction between tillage and fungicide on Powell amaranth emergence in 2016. In the absence of fungicide seed treatment, ST reduced emergence of Powell amaranth by approximately 50%, but this suppressive effect did not occur for fungicide treated seeds. This result suggests that part of the suppressive effect of ST in 2016 was due to increased fungal decay of seeds or young seedlings under ST compared to FWT.

Experiment 2: Ambient Weed Emergence. *Effects of tillage on emergence.* In 2015, tillage did not influence emergence of ambient populations of large crabgrass or Powell amaranth, but did effect common lambsquarters and carpetweed (Table 2.4). Common lambsquarters and carpetweed emergence were lowest in the ST+rye plots, regardless of herbicide application. However, within vetch treatments, carpetweed emergence was lower under FWT compared to ST. The greatest emergence of common lambsquarters occurred in the FWT+rye plots with no pre-emergence herbicide applied. In 2016, the effects of tillage on emergence of both Powell amaranth and carpetweed depended on the level of herbicide application (Table 2.4; significant tillage*herbicide interaction); when S-metolachlor was not applied, ST resulted in lower emergence of both species than FWT. However, when S-metolachlor was applied ST resulted in greater emergence of Powell amaranth compared with FWT.

Greater weed emergence following tillage may be explained in part by the stimulating effect of tillage on weed species germination (Mohler 2001). Since strip tillage leaves the between-row zone untilled, it is not surprising that ST without herbicide use reduced emergence for some species of this study. However, differences in emergence in this study

Table 2.4: Main effects, interactions, and overall ANOVA for ambient emergence of amaranth, crabgrass, lambsquarters, and carpetweed for 2015 and 2016

	Powell Amaranth		Large Crabgrass		Common Lambsquarters		Carpetweed	
	2015	2016	2015	2016	2015	2016	2015	2016
	number emerged / 0.25m^2							
Tillage Main Effect								
FWT	2.38 a	-	-	-	-	7.23 b	-	-
ST	1.00 a	-	-	-	-	39.56 a	-	-
Cover Crop Main Effect								
No cover crop	-	0.40 b	-	-	-	-	-	-
Rye	-	0.45 ab	-	-	-	-	-	-
Vetch	-	1.15 a	-	-	-	-	-	-
Herbicide Main Effect								
With herbicide	-	-	0.08 b	0.21 b	1.33 b	12.09 b	-	-
Without herbicide	-	-	1.05 a	3.34 a	5.69 a	30.26 a	-	-
Tillage x Herbicide Interaction								
FWT								
With herbicide	-	0.24 c	-	-	-	-	1.39 b	0.34 c
Without herbicide	-	2.37 a	-	-	-	-	18.65 a	26.90 a
ST								
With herbicide	-	0.94 b	-	-	-	-	4.45 b	0.12 c
Without herbicide	-	0.03 c	-	-	-	-	13.59 a	4.92 b
Tillage x Cover Crop Interaction								
FWT								
No cover crop	-	-	-	-	4.24 a	-	6.25 b	-
Rye	-	-	-	-	8.27 a	-	5.40 b	-
Vetch	-	-	-	-	3.59 ab	-	5.92 b	-
ST								
No cover crop	-	-	-	-	3.92 a	-	19.16 ab	-
Rye	-	-	-	-	0.18 b	-	0.25 c	-
Vetch	-	-	-	-	1.91 ab	-	27.15 a	-

Table 2.4 (cont'd)								
TillagexCover CropxHerbicide Inter.								
FWT								
No cover crop						-	-	
With herbicide	-	-	-	-	2.23 bcd	-	-	-
Without herbicide	-	-	-	-	6.89 abc	-	-	-
Rye						-	-	
With herbicide	-	-	-	-	3.47 bcd	-	-	-
Without herbicide	-	-	-	-	15.13 a	-	-	-
Vetch						-	-	
With herbicide	-	-	-	-	1.35 cd	-	-	-
Without herbicide	-	-	-	-	6.91 abc	-	-	-
ST								
No cover crop						-	-	
With herbicide	-	-	-	-	0.98 cd	-	-	-
Without herbicide	-	-	-	-	8.81 abc	-	-	-
Rye						-	-	
With herbicide	-	-	-	-	0.13 d	-	-	-
Without herbicide	-	-	-	-	0.25 d	-	-	-
Vetch						-	-	
With herbicide	-	-	-	-	1.12 cd	-	-	-
Without herbicide	-	-	-	-	2.91 bcd	-	-	-
ANOVA								
Tillage	+	+	N.S.	N.S.	N.S.	*	N.S.	***
Cover Crop	N.S.	+	N.S.	N.S.	N.S.	N.S.	**	N.S.
Herbicide	N.S.	N.S.	***	***	***	***	***	***
Tillage x Herbicide	N.S.	***	N.S.	N.S.	N.S.	N.S.	+	***
Tillage x Cover Crop	N.S.	N.S.	N.S.	N.S.	+	N.S.	**	N.S.
Cover Crop x Herbicide	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tillage x Cover Crop x Herbicide	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

likely also reflect differences in seedbank densities and vertical distribution of seeds in the surface layer resulting from long term tillage and cover crop treatments.

In 2016, emergence of ambient common lambsquarters was five times greater under ST compared to FWT regardless of cover crop or herbicide treatments (Table 2.4). This may indicate that, unlike amaranth and carpetweed, lambsquarters emergence was more influenced by long-term seedbank effects than short-term effects of tillage on germination. Since common lambsquarters germination is known to respond positively to germination cues such as light and nitrogen (Buhler 1997; Milberg et al. 1996; Sweeney et al. 2008), it is likely that higher emergence of common lambsquarters under ST reflects higher density of common lambsquarters in the surface germination zone of ST compared with FWT.

Effect of cover crops on emergence. In 2015, cover crops had little or no effect on emergence of ambient populations of Powell amaranth and large crabgrass, but did affect carpetweed and common lambsquarters (Table 2.4). For carpetweed, emergence was suppressed by rye, but only within strip tillage treatments. In addition, both common lambsquarters and carpetweed had the lowest emergence in ST+rye plots, indicating that emergence was reduced by winter rye but not vetch residues left on the surface. This effect may be due to differences between cover crop species in residue mulch thickness with the winter rye cover crop providing greater biomass at 4,840-6,040 kg/ha compared with hairy vetch at 1,640-2,120 kg/ha. Additionally, winter rye residues have known allelopathic effects (Barnes and Putnam 1983) that may inhibit weed emergence. Furthermore, differences in emergence of ambient carpetweed and common lambsquarters may lower initial density of these species in the weed seedbank in winter rye compared with vetch treatments.

In contrast with carpetweed and lambsquarters, emergence of ambient populations of Powell amaranth were not influenced by the rye cover crop in either year. However, in 2016, Powell amaranth had marginally significant (P=0.0851) greater emergence under a vetch cover crop compared to no cover crop plots. Stimulatory effects of vetch on weed emergence may reflect higher nitrogen availability in vetch plots (Tables 2.5 and 2.6). Many populations of Powell amaranth are known to respond positively to inorganic nitrogen concentrations (Brainard et al. 2006; Kumar et al. 2008).

Effect of tillage and cover crops on herbicide efficacy. Not surprisingly, S-metolachlor suppressed emergence of most of the ambient weeds evaluated in this study. Ambient large crabgrass (2015 and 2016) and common lambsquarters (2016) had lower emergence when exposed to S-metolachlor at the time of crop planting (Table 2.4). Carpetweed was also suppressed by S-metolachlor, although the effect varied slightly depending on tillage.

The efficacy of S-metolachlor depended on either tillage or cover crop treatments for common lambsquarters in 2015 (Table 2.4; tillage*cover crop*herbicide P=0.0406) and Powell amaranth in 2016 (tillage*herbicide interaction P<0.0001). Interestingly, S-metolachlor did not suppress Powell amaranth in 2015 and its effect in 2016 depended critically on the level of tillage: emergence was reduced under FWT but apparently stimulated under ST (Table 2.4).

In 2015, herbicide efficacy on the control of common lambsquarters was greatly reduced in plots with winter rye and vetch residues remaining on the surface (Figure 2.2). Winter rye and vetch residues incorporated into the soil by FWT did not reduce herbicide efficacy suggesting that S-metolachlor is only negatively impacted by residues left as a physical mulch barrier.

Table 2.5: Main effects, interactions, and overall ANOVA for soil ammonium and nitrate levels in 2015

	11-Jun		16-Jun		22-Jun	
	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
	ppm					
Tillage Main Effect						
FWT	-	-	7.62 a	-	11.90 a	41.58 a
ST	-	-	3.04 b	-	2.65 b	4.64 b
Cover Crop Main Effect						
No cover crop	-	-	3.65 b	-	-	-
Rye	-	-	4.58 b	-	-	-
Vetch	-	-	7.76 a	-	-	-
Tillage x Cover Crop Inter.						
FWT						
No cover crop	15.15 a	9.43 b	-	2.87 bc	-	-
Rye	17.26 a	10.84 b	-	2.33 c	-	-
Vetch	17.15 a	18.47 b	-	3.17 bc	-	-
ST						
No cover crop	3.86 b	19.73 b	-	2.66 bc	-	-
Rye	3.39 b	9.38 b	-	3.71 bc	-	-
Vetch	16.83 a	29.89 a	-	6.95 a	-	-
ANOVA						
Tillage	*	+	*	*	**	*
Cover Crop	+	**	*	***	N.S.	N.S.
Tillage x Cover Crop	+	+	N.S.	**	N.S.	N.S.

*Where different letters indicate significant differences between treatments at α=0.05 and the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Table 2.6: Main effects, interactions, and overall ANOVA for soil ammonium and nitrate levels in 2016

	1-Jun		9-Jun		16-Jun		21-Jun	
	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
	ppm							
Tillage Main Effect								
FWT	-	-	15.86 a	-	52.77 a	-	-	-
ST	-	-	6.29 b	-	10.12 b	-	-	-
Cover Crop Main Effect								
No cover crop	-	-	8.42 b	-	-	-	-	18.38 b
Rye	-	-	10.04 b	-	-	-	-	23.99 b
Vetch	-	-	14.76 a	-	-	-	-	32.73 a
Tillage x Cover Crop Interaction								
FWT								
No cover crop	2.26 d	3.12 c	-	3.79 c	-	14.33 bc	21.21 b	-
Rye	2.39 d	3.39 c	-	3.63 c	-	19.46 b	32.08 a	-
Vetch	3.64 bc	6.14 b	-	6.17 bc	-	21.13 ab	24.15 b	-
ST								
No cover crop	2.52 cd	5.87 b	-	5.34 bc	-	13.49 bc	4.67 dc	-
Rye	3.69 b	10.65 a	-	8.20 b	-	8.04 c	3.01 dc	-
Vetch	6.22 a	11.62 a	-	12.80 a	-	29.86 a	8.80 c	-
ANOVA								
Tillage	+	**	*	*	**	N.S.	**	N.S.
Cover Crop	***	***	***	***	N.S.	**	*	**
Tillage x Cover Crop	+	+	N.S.	*	N.S.	**	**	N.S.

*Where different letters indicate significant differences between treatments at α=0.05 and the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Our results demonstrated that the efficacy of S-metolachlor is sometimes reduced in conservation agricultural systems (Figure 2.2). However, cover cropping in combination with ST can also suppress emergence (Figure 2.1). The net effect of these contradictory effects is difficult to predict, but may help explain variation in results observed in previous studies. S-metolachlor is considered highly mobile (Sanchez-Martin et al. 1995) and may not bind to cover crop residue. It has been previously suggested that irrigation could be used to purposefully move herbicides through residue to the soil surface (Marble 2015) although too much irrigation could relocate the herbicide to below the weed seed germination zone.

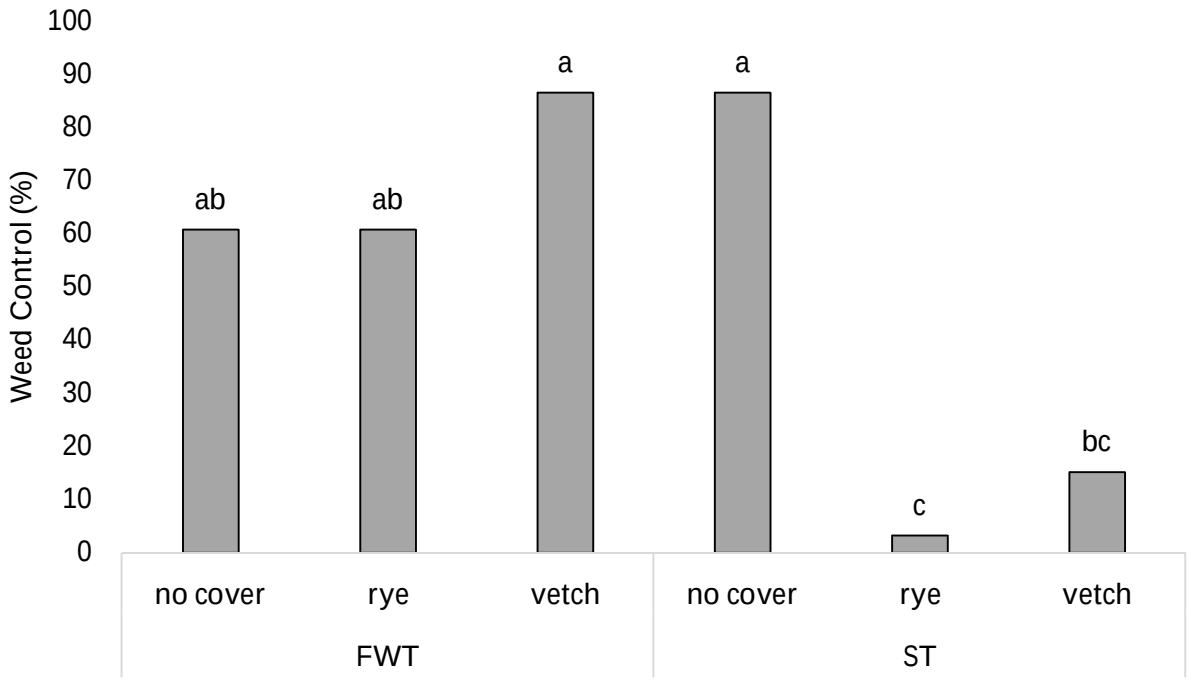

Figure 2.2: Tillage and cover crop effects on herbicide efficacy (% weed control) for common lambsquarters in 2015. Different letters indicate significant differences between treatments at α=0.05.

Ammonium and Nitrate. Both tillage and cover crops influenced nitrogen availability in several cases in both years (Tables 2.5 and 2.6). Soils from vetch cover crop plots had equal or significantly greater ammonium-nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) levels compared to all other treatments in both years. At sampling times when there were tillage by cover crop interactions, the highest levels were often in ST+vetch treatments (Tables 2.5 and 2.6). In addition, soils from FWT plots had significantly higher NH_4-N levels but lower NO_3-N levels compared with ST during the emergence periods of both 2015 and 2016 (Tables 2.5 and 2.6). NH_4-N levels were influenced by an interaction between tillage and cover crop, with the highest levels in FWT+rye and lowest in ST+no cover or ST+rye in 2016 (Table 2.6).

Inorganic nitrogen (both NH_4-N and NO_3-N) is known to stimulate emergence of some Powell amaranth populations (Brainard et al. 2006) and this effect may help explain higher emergence of ambient Powell amaranth in FWT treatments in 2015 and vetch treatments in 2016 (Table 2.4). However, no such vetch effect was observed for sown Powell amaranth seeds (Table 2.3), high inorganic nitrogen in other treatments (Tables 2.5 and 2.6) were not associated with higher emergence. These variable results suggest that N is not consistently the most important mechanism explaining Powell amaranth emergence responses to tillage and cover cropping. Previous studies have shown variable responses of Powell amaranth to N fertilizers (Kumar et al. 2008; Sweeney et al. 2008), due in part to intraspecific variation in N response (Brainard et al. 2006). It should be noted that amaranth seeds that were sown in our study did not originate from SWMREC and may have exhibited a different response to nitrogen than the ambient seeds.

Correlation analysis between total inorganic nitrogen and weed emergence showed that soil nitrogen levels during the early emergence period were positively correlated to the emergence of ambient lambsquarters and sown crabgrass as well as negatively correlated to the emergence of carpetweed (Table 2.7), although these relationships were not strong. Despite these significant correlations, the majority of analyses indicate that the emergence of sown seeds and ambient weeds were not well correlated with nitrogen levels in the soil (Table 2.7).

Table 2.7: Pearson's correlation coefficients between total inorganic nitrogen of soils from both years and weed emergence

		Emergence 19 DAP					
		Ambient Weeds				Sown Seeds	
		AMAPO	DIGSA	CHEAL	MOLVE	AMAPO	DIGSA
2015	11-Jun	0.1546	-0.1009	0.2478	0.3216	0.1148	**0.5999** **
	16-Jun	-0.0085	0.0539	0.0450	-0.1202	0.2795	0.3663 +
	22-Jun	-0.0914	0.1682	0.1404	-0.2487	0.2452	0.2926
2016	1-Jun	-0.3050	0.1909	**0.4082** *	**-0.6243** **	-0.1654	0.3008
	9-Jun	0.0476	-0.1810	-0.0490	-0.1233	0.1186	0.0577
	16-Jun	0.3761 +	-0.3417	-0.3353	**0.5291** **	0.1762	-0.2860
	21-Jun	0.2779	-0.3848 +	-0.2275	0.1585	-0.0918	-0.4019 +

*Where the above r^2 coefficients are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Soil Moisture and Temperature. Soil temperature ranges during the emergence periods of 2015 and 2016 were very similar (Figure 2.3) while soils in 2016 had lower moisture than those in 2015 (Figure 2.4). This suggests that differences in emergence seen between years were likely not influenced by soil temperature but may have been influenced by soil moisture. During the emergence period there were no initial differences in soil gravimetric water content (GWC) between tillage or cover crop treatments in 2016 (Table 2.8). Over time there were higher soil

moisture in ST plots with either winter rye or vetch cover crops. At the end of the emergence period soil moisture remained highest in winter rye plots compared with vetch treatments. Despite these plots having greater moisture, sown Powell amaranth and large crabgrass tended

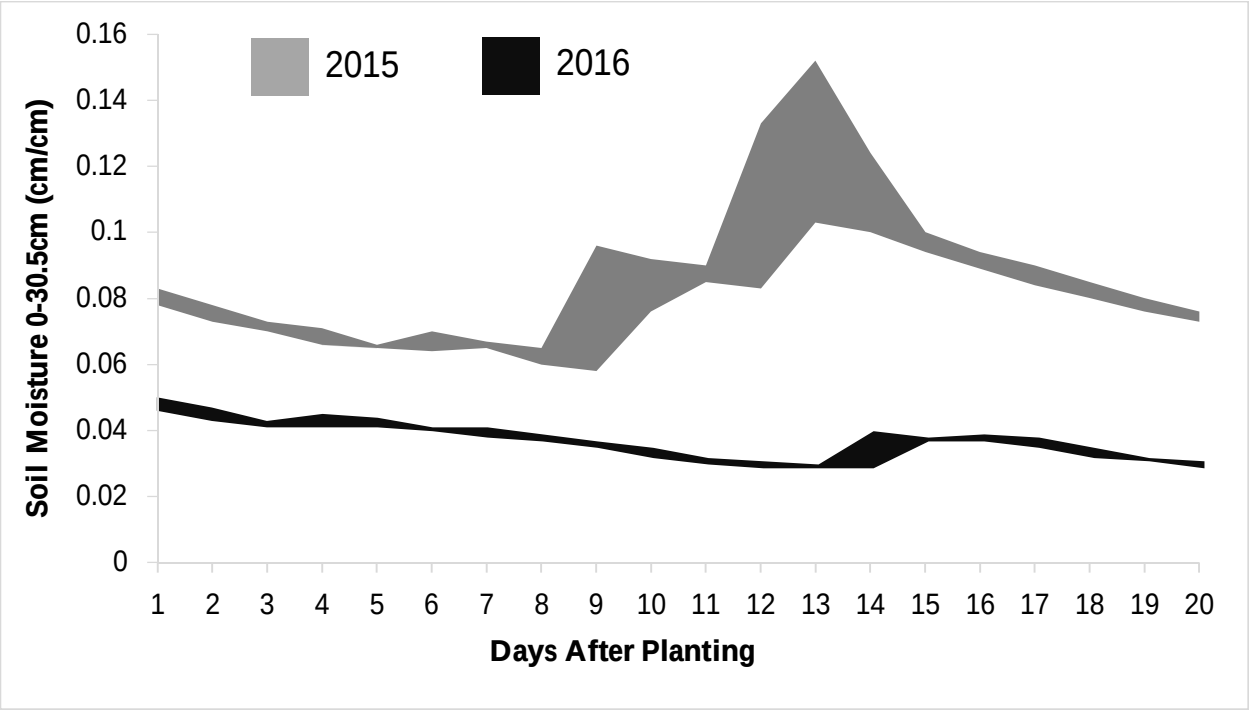

Figure 2.3: General soil moisture range at the Southwest Michigan Research and Extension Center during the weed emergence periods of 2015 and 2016.

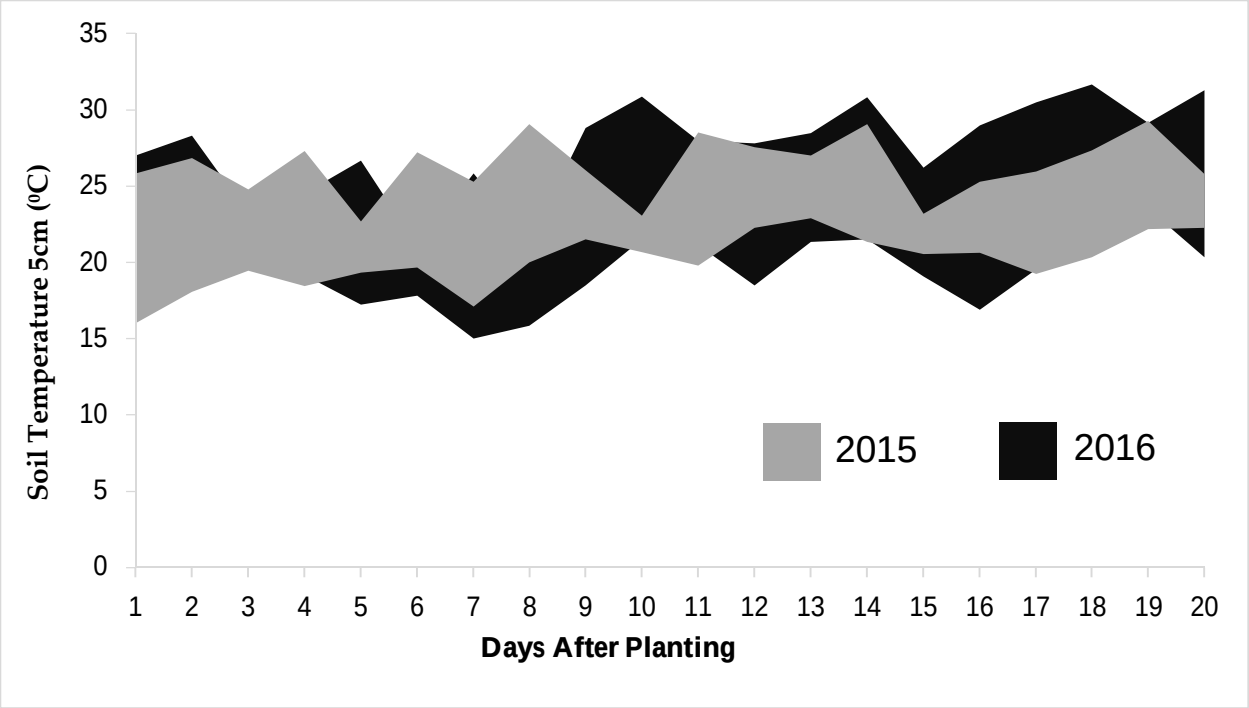

Figure 2.4: General soil temperature range at the Southwest Michigan Research and Extension Center during the weed emergence periods of 2015 and 2016.

Table 2.8: Main effects, interactions, and overall ANOVA for soil gravimetric water content in 2016

	9-Jun	16-Jun	21-Jun
	(g wet soil - g dry soil) / g dry soil		
Tillage Main Effect			
FWT	-	0.0613 b	0.0020 a
ST	-	0.0785 a	0.0029 a
Cover Crop Main Effect			
No cover crop	-	0.0653 b	0.0021 b
Rye	-	0.0739 a	0.0030 a
Vetch	-	0.0705 a	0.0023 b
Tillage x Cover Crop Interaction			
FWT			
No cover crop	-	0.0604 c	0.0020 b
Rye	-	0.0614 c	0.0021 b
Vetch	-	0.0621 c	0.0021 b
ST			
No cover crop	-	0.0701 c	0.0022 b
Rye	-	0.0865 a	0.0039 a
Vetch	-	0.0789 b	0.0025 b
ANOVA			
Tillage	N.S.	*	+
Cover Crop	N.S.	**	*
Tillage x Cover Crop	N.S.	**	+

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

to have lower emergence under ST and a winter rye cover crop. This may have resulted from soil temperature and inorganic nitrogen levels having greater impacts than soil moisture on seed germination, or that seed germination mainly occurred early during the emergence period when soil moistures were not different between treatments.

In 2016, daytime soil temperatures were higher for ST+rye plots compared to other treatments at 14 DAP but starting at 16 DAP ST+rye plots tended to have lower temperatures by several degrees (Table 2.9). Sown Powell amaranth and large crabgrass had lower emergence under ST and a winter rye cover crop which is consistent with seed sensitivity to warm

temperatures for germination. However, correlation analyses do not indicate that there is a significant relationship between soil temperature and either sown seed emergence or ambient weed emergence (Table 2.10).

Table 2.9: Main effects, interactions, and overall ANOVA for 2016 soil temperatures starting 14 DAP

	16-Jun		17-Jun	18-Jun		19-Jun		20-Jun		21-Jun	
	14 DAP		15 DAP	16 DAP		17 DAP		18 DAP		19 DAP	
						^{0}C					
Tillage Main Effect											
FWT	-		-	-		-		-		-	
ST	-		-	-		-		-		-	
Cover Crop Main Effect											
No cover crop	19.7	b	29.5	33.2		35.6	a	-		34.0	
Rye	20.0	a	28.5	31.5		33.7	b	-		32.5	
Tillage x Cover Crop Interaction											
FWT											
							a				a
No cover crop	19.8	b	-	32.7	a	34.9	b	32.3	a	33.4	b
		a					a				a
Rye	19.8	b	-	32.6	a	34.8	b	32.1	a	33.3	b
ST											
No cover crop	19.6	b	-	33.7	a	36.2	a	32.9	a	34.6	a
Rye	20.2	a	-	30.4	b	32.7	b	30.3	b	31.8	b
ANOVA											
Tillage	N.S.		N.S.	N.S.		N.S.		N.S.		N.S.	
Cover Crop	*		+	*		*		*		+	
Tillage x Cover Crop	+		N.S.	*		+		*		+	

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Table 2.10: Pearson's correlation coefficients between soil temperatures and ambient weed emergence or sown seed emergence

	Ambient Weed Emergence				Sown Seed Emergence	
	AMAPO	DIGSA	CHEAL	MOLVE	AMAPO	DIGSA
16-Jun day	-0.2852	0.2167	-0.2193	-0.2762	-0.2653	-0.2334
16-Jun night	-0.2241	0.3266	-0.0915	-0.2679	-0.2717	-0.1993
17-Jun day	0.3639	-0.2770	0.0376	0.2978	0.2700	0.3715
17-Jun night	0.1677	0.2420	0.0442	0.2008	-0.4101	0.0034
18-Jun day	0.3221	-0.2818	0.0811	0.2513	0.3283	0.3417
18-Jun night	0.1646	-0.0098	-0.0580	0.2853	-0.1064	-0.1558
19-Jun day	0.2852	-0.2894	0.1025	0.2121	0.3376	0.3649
19-Jun night	0.1945	-0.1994	-0.0624	0.3556	0.0303	-0.1425
20-Jun day	0.2987	-0.3981	0.0560	0.3693	0.4386 +	0.2651
20-Jun night	0.0644	-0.0873	-0.2453	0.2153	-0.1238	-0.3524
21-Jun day	0.2892	-0.3188	0.1076	0.1862	0.3573	0.3815
21-Jun night	0.1024	-0.2495	-0.2568	0.3476	-0.0832	-0.2965

*Where the above r^2 coefficients are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Summary and Conclusions

The primary objectives of our research were to evaluate how tillage and cover crops influence the emergence of summer annual weeds and whether these effects are mediated by fungal pathogens and herbicide efficacy. We had hypothesized that, in the absence of herbicides, sown weed emergence would be lower in ST compared to FWT and lower in cover crop compared to no cover crop treatments. We found in 2015 that in the absence of herbicides there was lower weed emergence in ST+rye for both Powell amaranth and large crabgrass, supporting our hypothesis. In the second year, amaranth had lower emergence in ST while crabgrass emergence was lower in cover crop plots. These results are consistent with other studies that have shown lower Powell amaranth emergence under no-till compared with conventional tillage (e.g. Peachey et al. 2004).

We found little support for the hypothesis that the suppressive effects of strip tillage and cover cropping were mediated by fungal pathogens. However, Powell amaranth emergence in 2016 was suppressed by ST only when seeds were unprotected by fungicide treatment (Table 2.3). Although this effect was only marginally significant (P=0.0919), it suggests that fungal pathogens may strongly influence tillage effects on emergence in some cases. This is perhaps not surprising given that tillage influenced soil moisture (Table 2.8) and nitrogen dynamics (Table 2.6), both of which can impact the abundance of fungal pathogens like *Pythium* (Frey et al. 1999) which have known negative effects on weed emergence.

Other factors possibly mediating tillage and cover crop effects included soil temperature, soil moisture, and soil inorganic nitrogen. Soils from ST and winter rye cover crop plots tended to have cooler temperatures, lower levels of ammonium-nitrogen, and greater

moisture content. These treatments also had lower sown seed emergence, which is not surprising given that Powell amaranth emergence increases as temperatures increase (Weaver et al. 1988) and at greater nitrogen availability. However, given that Powell amaranth emergence has been seen to increase as soil moisture increases (Weaver et al. 1988), it is surprising that we saw lower emergence where soil moisture was higher. This may indicate that soil temperature and the available nitrogen had a greater influence than moisture during these trials, or that higher soil moisture affected Powell amaranth emergence indirectly through increases in fungal mediated post-germination mortality.

Lastly we had hypothesized that the efficacy of S-metolachlor might be reduced under ST treatments with cover crops, resulting in higher emergence of species sensitive to this herbicide. This hypothesis was partly supported by our finding that the efficacy of S-metolachlor on both common lambsquarters and Powell amaranth was sometimes reduced in conservation agricultural systems (Table 2.4 and Figure 2.2). However, the practical implications of this finding are unclear; even when herbicide efficacy was reduced, cover cropping and reduced tillage resulted in equivalent or lower net emergence.

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CHAPTER THREE: Tillage and Cover Crop Effects on Summer Annual Seed Persistence

Abstract

Weed seedbank density and composition in intensive vegetable production systems may shift when tillage is reduced or cover crops are used. We hypothesized that reduced tillage and rye cover cropping would influence seed persistence, and that differences in persistence could be explained in part by fungal pathogens and exposure to light and oxygen. To test these hypotheses, fungicide treated (captan, trifloxystrobin and metalaxyl) and untreated seeds of Powell amaranth and large crabgrass were buried in October in mesh bags between crop rows in a long term vegetable cropping system experiment with two tillage treatments (full width tillage [FWT] or strip tillage [ST]) and two cover crop treatments (none or winter rye). Tillage and cover crop treatments had been imposed on the same plots for six years prior to burial in a sweet corn-snap bean-cucurbit rotation on sandy soils in southwest Michigan. Bags were exhumed in December, March, June, July, and September (1.0, 4.5, 7.0, 8.5, and 10.5 months after burial) and seeds were tested for viability using 2,3,5-triphenyl tetrazolium chloride. At the June sampling date, immediately prior to spring tillage in the FWT no-cover crop plots, a subset of weed seed bags were exhumed in light, another set in the dark, and a third set under low levels of oxygen to determine if exposure to light or atmospheric levels of oxygen influenced weed seed persistence. These bags were reburied after tillage was complete and seed viability was then evaluated in July. After eight and a half months of burial, Powell amaranth and large crabgrass seeds in ST had two to three times greater persistence than those in FWT. Large crabgrass seeds had two-fold greater persistence under winter rye cover cropping compared to

no cover crop. There was no evidence that differences in Powell amaranth or large crabgrass persistence were related to fungal pathogens or exposure to high levels of oxygen. In contrast, light exposure appeared to be a factor explaining reduced persistence of both species in FWT compared to ST. These results demonstrate that reduced tillage and cover cropping practices aimed at improving soils qualities may increase seed persistence of weed species.

Nomenclature. Powell amaranth, *Amaranthus powellii* S. Wats. AMAPO; large crabgrass, *Digitaria sanguinalis* (L.) Scop. DIGSA; winter rye, *Secale cereale*.

Key Words. Strip-tillage, fungicide, captan, trifloxystrobin, metalaxyl, seed viability, seed burial, light exposure, oxygen exposure.

Introduction

Emergence and Persistence. In chapter 2 we addressed the effects of tillage and cover crops on summer annual emergence. We saw that following long-term tillage and cover cropping ambient weed emergence was greater under ST than FWT and greater under a rye cover crop than no cover crop. However, when we controlled the density and vertical distribution of weed seeds by sowing known quantities at a uniform depth we observed the opposite trend: emergence was lower under ST compared with FWT and lower under a rye cover crop than no cover crop. These results correspond well with the concept that, in the short-term, tillage stimulates germination and thus emergence, while cover crops provide physical, chemical or biological inhibition of germination and emergence. The reason for greater emergence in conservation agricultural systems in the long-run are less clear but likely reflect greater seedbank densities in the germination zone due to some combination of greater seed production, greater concentration of seeds near the soil surface, and greater persistence of seeds.

Seed Persistence. Relatively little information is available on the potential effects of conservation agriculture practices such as cover crops and strip tillage on seed persistence. The persistence of weed seeds in the soil is influenced by many factors including temperature, moisture, the presence of predators, and the presence of pathogens (Long et al. 2015; Schafer and Chilcote 1970). In preliminary studies at SWMREC, winter rye and rye-vetch cover crops increased the longevity of Powell amaranth relative to no cover in strip-tillage systems (Brainard, unpublished data).

Differences in seed persistence may reflect differences in the rates of seed decay, predation or germination. Several mechanisms encourage seed germination in response to a tillage event including light exposure, increased or fluctuating soil temperature, increased soil aeration, and increased nitrogen mineralization (Mohler 2001). Strip-tillage reduces tillage and in these systems weed emergence is often reduced (Brainard and Noyes 2012; Hendrix et al. 2004; Wang and Ngouajio 2008). Greater persistence in ST compared to conventional tillage may be due in part to lower rates of withdrawal because of less light exposure. In addition, tillage and cover crop residues may alter persistence through changes in temperature, moisture and other soil characteristics influencing seed decay or germination, or through changes in predator habitat that influence rates of predation.

Light Exposure. The impact of tillage-induced light exposure on germination of annual weeds is well known. Previous research indicates that light exposure influences seed germination for many weed species (Wesson and Wareing 1967). For example, studies have found that red light pulses and brief light exposure increased germination of a crabgrass species (Tang et al. 2010), common lambsquarters (Milberg et al. 1996) and many species in the Amaranthus genus closely related to Powell amaranth (Liebman et al. 2001). Several studies have demonstrated that tillage or cultivation events occurring at night results in lower rates of emergence for some, but not all, weed species (Botto et al. 1998; Buhler 1997; Fogelberg 1999). Brief light exposure after a period of burial has increased the germination of the summer annual weed *Datura ferox* (Scopel et al. 1991). This study also found that *D. ferox* germination response to soil disturbance was light-

dependent and that light exposure was the only requirement for triggering germination in the field.

Despite the well-known impact of light exposure on seed germination, surprisingly few studies have quantified the importance of light exposure and subsequent fatal germination on seed persistence under conservation tillage. For light sensitive species that show greater emergence following day-time cultivation (Scopel et al. 1994), conservation agriculture practices such as ST or cover crop use may reduce seed germination and therefore increase seed persistence.

Objectives and Hypotheses. Since managing the weed seedbank is an important part of successful production systems it is import to understand how conservation agriculture practices influence the persistence of seeds within the seedbank. Improved understanding of the population dynamics of important weed species may help in identification of practices that most efficiently and economically disrupt their life cycles. The objectives of this study were to evaluate the effects of tillage and winter rye cover cropping on weed seed persistence, and to investigate the extent to which these effects are mediated by fungal pathogens and exposure to light or oxygen. We hypothesized that 1) weed seed persistence would be lower under ST compared to FWT and lower in winter rye cover crop compared to no-cover crop, 2) the effects of cover crops and tillage on seed persistence would differ between fungicide treated and untreated seeds, and 3) tillage effects on weed seed persistence would be due to the stimulating effects of light and oxygen on seed germination.

Materials and Methods

Long-term Trial Experimental Treatments and Design. Two weed seed burial experiments (Experiment 1 and Experiment 2) were conducted within a subset of treatments in a long-term tillage trial initiated in September 2008 on Oakville fine sand at the Southwest Michigan Research and Extension Center in Benton Harbor, Michigan (42.085244^0 N, 86.358736^0 W). Experimental treatments included all combinations of three factors: tillage (strip-tillage [ST] vs. conventional full-width tillage using a moldboard plow [FWT]), cover crop (no cover crop [no cover], winter rye [rye], or either a hairy vetch winter rye mix (until 2014) or hairy vetch (since 2014)), and weed management (reduced input [low] vs. conventional grower practice [high]). Treatments were imposed in the same plots each year with crops following a three year rotational sequence of sweet corn-snap bean-cucurbit crop (butternut squash in 2011 and pickling cucumber in 2014). Plots were arranged in a split-split plot design with tillage as the main plot factor, cover crop as the sub-plot factor, and weed management as the sub-sub plot factor. Tillage main plots measured 11.4m x 18.3m and were arranged in a randomized complete block design with four replications. Weed management split-split plots were 3.8m x 9.1m with either two (winter squash) or five rows (all other crops) per plot.

Seed burial Experiments 1 and 2 were conducted only in the low weed management sub-sub plots of this long-term experiment to avoid any confounding effects of historical weed management intensity on seed persistence. In addition, only the rye and no cover crop control plots were included. Therefore, only the details of these treatments will be described.

Field Management. Winter rye was drilled at 125kg/ha in mid-September of 2015 using a grain drill with 19cm between-row spacing. The following spring tillage occurred in May or early June depending on the crop. Full width tillage consisted of moldboard plowing followed by disking and field cultivating. Strip-tillage was accomplished using either a Hiniker 6000 strip-tiller (for sweet corn, snap beans, and cucumbers) or an Unverferth 120 subsoiler (for winter squash). Both strip-tillage implements were equipped with a row-cleaner (to remove cover crop residue), a shank, offset disks, and a rolling basket. Strip tillage resulted in an approximately 25cm wide by 30cm deep zone of disturbed soil into which crops were planted.

Weed management in the low intensity treatments varied by crop, and included both herbicides and mechanical cultivation. In snap beans and sweet corn, herbicides included S-metolachlor (Dual Magnum, 1 pint/acre) pre-emergence with a post-emergence application of sodium salt of bentazon (Basagran, 0.75quarts/acre) and fomesafen sodium salt (Reflex, 0.5 pint/acre). In some years, snap beans also received a post-emergence application of clethodim (SelectMax, 1 pint/acre) to control grass weeds as needed. For cucurbit crops, herbicides included a pre-emergence application of ethalfuralin/clomazone (Strategy, 3 pints/acre) and a post-emergence application of clethodim (SelectMax, 1 pint/acre). In FWT treatments, cultivation with s-tine sweeps was also used as needed to manage weed escapes between crop rows. Rates and timings of herbicide applications differed slightly by year and crop but were identical for all treatments within a given year.

Experiment 1: Tillage and Cover Crop Effects on Seed Persistence. *Experimental design.* This experiment evaluated the effects of tillage (ST vs. FWT), cover crop (no cover vs. rye) and seed

treatment (fungicide treated vs. untreated) on seed persistence of Powell amaranth (*Amaranthus powellii* S. Wats., AMAPO, collected in 2011 from Hickory Corners, MI) and large crabgrass (*Digitaria sanguinalis* (L.) Scop., DIGSA, collected in 2012 from Benton Harbor, MI). Seeds were buried only within low weed management sub-sub plots of the long-term trial described above (Table 3.1). Therefore, for each weed species the design was a split-split plot design with tillage as the main plot factor, cover crop as the sub-plot factor, and fungicide as the sub-sub plot factor.

Table 3.1: Trial design and treatments

Factor	Treatment	Levels
Experiment One		
Whole plot	tillage	strip-till, conventional moldboard plow
Sub plot	cover crop	no cover crop, winter rye
Sub-sub plot	fungicide	triple-fungicide coating, no fungicide
Experiment Two		
Whole plot	tillage	conventional moldboard plow
Sub plot	cover crop	no cover crop
Sub-sub plot	exhumation conditions	light exposure, no light exposure, reduced oxygen exposure

Seed preparation. A subset of seeds from both species were coated with a triple fungicide treatment used in previous studies to protect weed seeds against fungi including *Rhizoctonia*, *Fusarium*, *Pythium*, and *Phytophthora* (Kumar et al. 2008, 2011). This coating contained captan (Captan Fungicide, 71 mg ai/100 g seed, Southern Agricultural Insecticides, Inc.), trifloxystrobin (Flint, 10 mg ai/100 g seed), and metalaxyl (Apron 35 SD, 15 mg ai/100 g seed). Untreated seeds were from the same seed lot but received no fungicide coating. Seed germination testing

revealed that fungicide treatment influenced germination of Powell amaranth seeds in petri dishes, although this was not consistently significant across multiple trials. For Powell amaranth, fungicide treated seeds had approximately 11% higher germination, but only in the light. Large crabgrass seeds did not respond to fungicide treatment regardless of light condition when germinated in petri dishes.

Seed burial. For each species and fungicide treatment combination, 100 seeds were mixed with 125g of white silica sand and placed in noseeum mesh bags. Silica sand was used for ease of subsequent seed separation and because it mimicked the soil texture at our experimental site (94% sand). In October of 2015 after cover crop planting (Table 3.2), seed bags were buried at a depth of 10.2cm in all four cover crop x tillage treatments (FWT+no cover, FWT+ rye, ST+no cover, FWT+rye). For bags containing seeds not receiving fungicide treatment, eight bags of each species were buried in each of the 16 cover crop sub-plots so that two bags per plot could be removed for each of four subsequent exhumation dates. For bags containing fungicide treated seeds, four bags of each species were buried in each plot so that two bags could be pulled at two subsequent exhumation dates in order to assess fungicide effects. Only two exhumation dates were evaluated for fungicide effects because of the likely limited persistence of fungicides themselves. Bags were buried by using a golf-cup hole cutter to remove a cylindrical soil core (10.2cm deep with a 11.4cm diameter), placing a single seed bag in the hole, replacing the soil core and tamping with moderate pressure so that the surface of the core was level with the surrounding soil. In treatments containing rye surface residue, the residue was carefully removed prior to burial and replaced after burial. Bags were connected to metal washers on the soil surface for easy identification and removal. In FWT treatments, all seed bags

(other than those used in Experiment 2) were retrieved in the morning before tillage, placed in paper bags, stored in a cooler at 4^{0}C, and reburied that same afternoon after tillage operations were complete. This process was necessary to avoid disturbance of the seed bags during tillage. The day of tillage was partly cloudy with a high temperature of 26.1^{0}C and the duration of bag storage was six hours.

Table 3.2: Timing of relevant field operations and experimental procedures.

Date	Field Operation	Experiment Operation
2015		
Aug-24	sweet corn harvested	
Sept-4	corn residue disked	
Sept-10	rye seeded	
Oct-27		weed seed bags buried, soil samples collected
Dec-1		exhumation 1, soil samples collected
2016		
Mar-22		exhumation 2, soil samples collected
May-19	rye cover terminated	
Jun-1	tillage	exhumation 3, soil samples collected, all bags removed and reburied in conventional-till
Jun-2	snap beans planted	
Jun-10	overhead irrigation installed	
Jul-27		exhumation 4, soil samples collected
Jul-28	beans harvested	
Aug-17	bean residue disked	
Aug-23	rye seeded, 2 bushel/acre	
Sept-8		exhumation 5, soil samples collected

Seed retrieval and viability assessment. Seed bags for DIGSA were retrieved after one month (with and without fungicide), four and a half months (with and without fungicide),

seven months (without fungicide) and nine months (without fungicide). Bags for AMAPO were retrieved after one month (with and without fungicide) seven months (with and without fungicide), nine months (without fungicide) and 10.5 months (without fungicide). Upon retrieval all bags were placed in cold storage at 4^0C until processing to evaluate seed viability. Seeds were separated from silica sand using a 600 micron sieve and tested for germination in 9cm petri dishes using No. 1 Whatman filter paper saturated with either 2mL distilled water for DIGSA or 2mL 0.002M gibberellic acid for AMAPO. These seeds were placed in a 16hr day/8hr night growth chamber set at 30^0C/25^0C that provided up to 28$\mu molm^{-2}s^{-1}$ of light. These treatments and conditions were chosen based on the stimulation of high germination rates in preliminary studies with non-buried seeds from the same seed lots. After two weeks all un-germinated seeds were tested for viability using a 0.1% 2,3,5-tretrazolium chloride (TZ) solution in accordance to methods outlined by The Tetrazolium Subcommittee of the Association of Official Seed Analysts in the 2000 revised handbook.

Statistical analysis. The total number of viable seeds for each seed bag for each exhumation date t (Nv,t) was calculated according to the equation:

$$(1) \qquad Nv,t = (Ng,t + Ntz,t)$$

where Ng,t is the total number of seeds retrieved at a time t that germinated in the growth chamber post-exhumation and Ntz,t is the total number of seeds retrieved at time t that did not germinate, but tested TZ positive. The proportion of viable seeds for each exhumation date t (Pv,t) was then calculated according to:

(2) $P_{v,t} = N_{v,t}/N_{r,t}$

where $N_{r,t}$ is the total number of seeds that were recovered at exhumation date t. The proportion of persistent seeds at a given exhumation date t ($P_{p,t}$) was defined as:

(3) $P_{p,t} = N_{v,t}/N_{v,1}$

where $N_{v,1}$ is the total number of viable seeds at the December exhumation time (t=1). Persistence was defined based on the December time point because seeds would have become acclimated to the environment rather than seed storage conditions. Mean values of these responses from the two bags recovered from each sub-sub plot were used for subsequent analysis.

For the 1, 4.5 (DIGSA only), and 7 month (AMAPO only) exhumation dates, the effects of tillage, cover crop and fungicide treatment on $N_{V,t}$, $P_{v,t}$ and $P_{p,t}$ were analyzed using the PROC GLIMMIX procedure in Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC). At these exhumation dates, data were analyzed as a split-split plot design with tillage, cover crop and fungicide treated as fixed effects, and replicate, replicate x tillage, and replicate x tillage x cover crop as random effects. For the 7 (DIGSA only), 8.5, and 10.5 month (AMAPO only) exhumation dates, where fungicide treatments were not included, data was analyzed as a split-plot design with tillage and cover crops as fixed effects and replicate and replicate x tillage as random effects. Where main or interactive effects were significant, treatment mean separation occurred using Fisher's Protected LSD at α=0.05.

Experiment 2: Role of Light and Oxygen on Tillage-Mediated Seed Persistence. *Experimental design.* To evaluate the potential impact of light or oxygen (O_2) exposure on seed persistence, three exhumation procedures were evaluated for seeds buried in no cover crop subplots within FWT main plots: 1) exhumation as described for Experiment 1 above (ambient light and O_2 exposure); 2) exhumation in darkness (no light but ambient O_2 exposure); and 3) exhumation underwater (light exposure but limited O_2 exposure) (Table 3.1). To accommodate these treatments, four additional bags of untreated AMAPO and DIGSA seeds were buried in all four FWT+no cover plots to allow for two bags to be evaluated for each of the additional exhumation procedures at one subsequent exhumation date.

Exhumation, storage, and reburial. For seed bags without exposure to light, an opaque plastic box with gloved openings was placed upside-down over the soil. The edges of the box were buried carefully to exclude all light while bags were removed and placed in sealed tins contained within the box. Seeds were stored in tins at 4^{0}C for eight hours in the same location as seed exposed to light. Reburial was managed by again excluding light using the box. For seed bags retrieved with minimal exposure to oxygen, seed bags were exhumed, immediately sealed in clear plastic bags, and submerged underwater to minimize gas exchange. During this process, gradual leakage occurred resulting in seed bags that were saturated with water. As with other treatments, these low O_2 seed bags were kept in cold storage for six hours and reburied following tillage.

Retrieval and viability testing. All bags for this experiment were left in the field for 1.5 months after reburial at which point they were removed and tested for viability as described in Experiment 1 above.

Statistical analysis. The effects of exhumation procedure on Nv,t, and Pv,t (see equations 1 and 2) were analyzed using the PROC GLIMMIX procedure in Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC) with exhumation procedure as a fixed effect and replicate as a random effect. The proportion of persistence seeds were calculated as:

$$(4) \qquad Pv,4b = Nv,4/Nv,3$$

where Nv,3 is the total number of viable seeds at the June exhumation time (t=3). Persistence for experiment 2 was defined based on the June time point in order to better evaluate seed persistence specifically after light and oxygen exposure during the tillage event. Mean values of these responses from the two bags recovered from each sub-sub plot were used for subsequent analysis.

Experiment 1: Tillage and Cover Crop Effects on Seed Persistence. *Effects of tillage on persistence.* Powell amaranth seed viability and persistence were influenced by tillage, but not cover crop (Table 3.3). This tillage effect was only evident after eight and a half months of burial and only after removal and reburial to accommodate spring tillage in the FWT plots (Table 3.3; Figure 3.1). The proportion of persistent amaranth seeds recovered in ST treatments was not greatly changed between June and September following tillage (Figure 3.1). In contrast, the proportion of persistent amaranth seeds in FWT treatments declined by approximately 60% during the same time period (Figure 3.1). These results do not support our hypotheses that persistence would be lower under ST compared with FWT and lower under a winter rye cover crop compared with no cover crop. Persistence differences between tillage treatments may be the result of tillage-based changes in factors including soil temperature, soil moisture, oxygen availability, light exposure, or fungal pathogens. The potential role of several of these factors will be addressed later in this chapter.

Table 3.3: Main effects, interactions, and overall ANOVA for the proportion of persistent Powell amaranth seeds.

	JUNE-2016 7 months	JULY-2016 8.5 month	SEPT-2016 10.5 months
		$N_{v,t}/N_{v,1}$	
Tillage Main Effect			
FWT	0.85	0.31 b	0.35 b
ST	0.88	0.78 a	0.88 a
Cover Crop Main Effect			
No Cover	0.82	0.61	0.55
Rye	0.91	0.48	0.68
Fungicide Main Effect			
With fungicide	0.90	n.a.	n.a.

Table 3.3 (cont'd)

Without fungicide	0.83	n.a.	n.a.
ANOVA			
Tillage	N.S.	*	**
Cover Crop	N.S.	N.S.	N.S.
Fungicide	N.S.	n.a.	n.a.
Tillage x Cover Crop	N.S.	N.S.	N.S.
Tillage x Fungicide	N.S.	n.a.	n.a.
Cover Crop x Fungicide	N.S.	n.a.	n.a.
Tillage x Cover Crop x Fungicide	N.S.	n.a.	n.a.

*Where n.a. indicates that the given effect or interaction is not applicable for that date and the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.)*

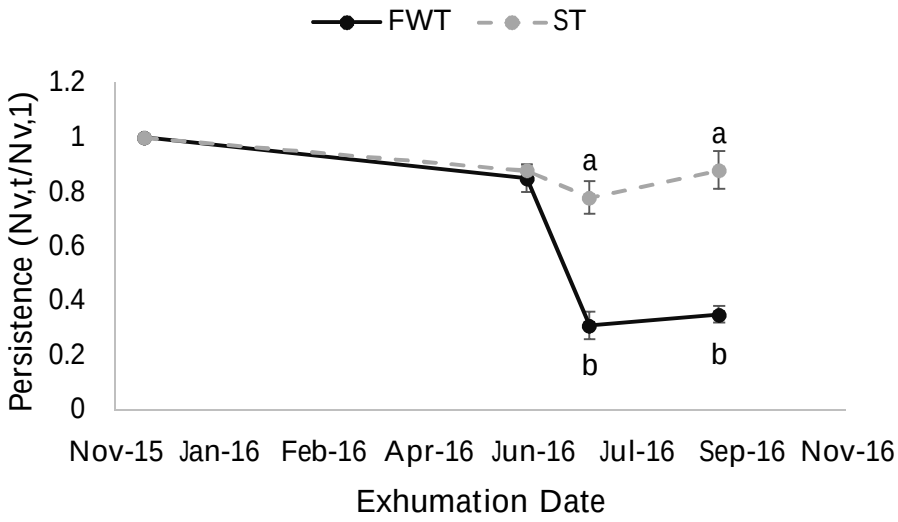

Figure 3.1: Tillage effect on proportion persistent (mean ± SE) Powell amaranth between December and 10.5 months after burial. Different letters indicate significant differences between tillage treatments. Means were separated using Fisher's Protected LSD at α=0.05.

Large crabgrass viability was influenced by both tillage and cover crop (Table 3.4). As with Powell amaranth, the persistence of large crabgrass seeds was greater in ST compared to FWT treatments, but only following removal and reburial to accommodate spring tillage

(Figure 3.2). Specifically, seed persistence declined by 63% between December and July is ST plots compared to 88% in FWT treatments. Contrary to our hypothesis, persistence was lower under ST compared with FWT.

Table 3.4: Main effects, interactions, and overall ANOVA for the proportion of persistent large crabgrass seeds.

	MAR-2016 4.5 months	JUNE-2016 7 months	JULY-2016 8.5 months
	Nv,t /Nv,1		
Tillage Main Effect			
FWT	0.51	0.55	0.12 b
ST	0.61	0.61	0.37 a
Cover Crop Main Effect			
No Cover	0.41 b	0.42 b	0.17
Rye	0.72 a	0.75 a	0.31
Fungicide Main Effect			
With fungicide	0.57	n.a.	n.a.
Without fungicide	0.56	n.a.	n.a.
ANOVA			
Tillage	N.S.	N.S.	*
Cover Crop	**	**	N.S.
Fungicide	N.S.	n.a.	n.a.
Tillage x Cover Crop	N.S.	N.S.	N.S.
Tillage x Fungicide	N.S.	n.a.	n.a.
Cover Crop x Fungicide	N.S.	n.a.	n.a.
Tillage x Cover Crop x Fungicide	N.S.	n.a.	n.a.

*Where n.a. indicates that the given effect or interaction is not applicable for that date and the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.)*

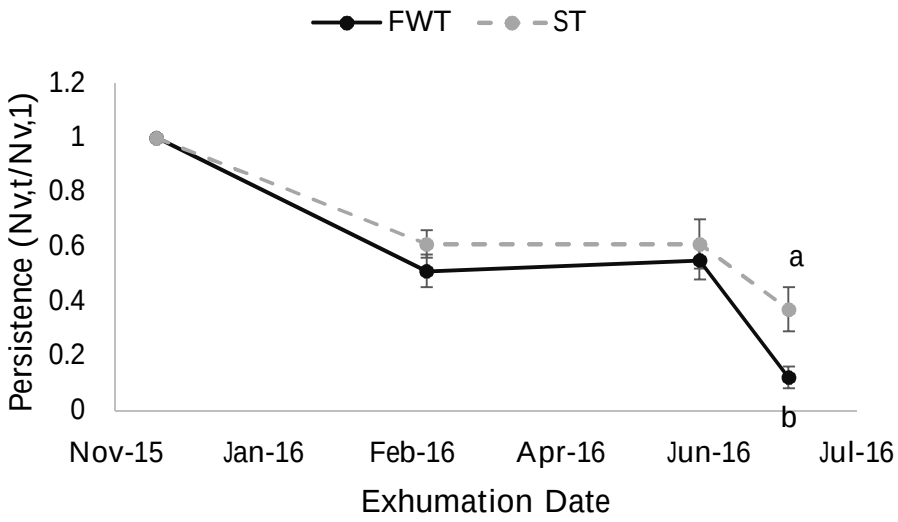

Figure 3.2: Tillage effect on proportion persistent (mean ± SE) large crabgrass between December and 10.5 months after burial. Different letters indicate significant differences between tillage treatments. Means were separated using Fisher's Protected LSD at α=0.05.

Our finding that seeds of both Powell amaranth and large crabgrass had greater persistence under ST compared with FWT is consistent with several other studies demonstrating greater persistence of summer annual weeds under no-tillage conditions compared with conventional full-width tillage (Davis et al. 2005; Steckel et al. 2007). Although weed seedbank dynamics under ST are in theory different from those under no-till due to greater spatial heterogeneity (Brainard et al. 2013), in practice they may be quite similar.

Effects of cover crops on persistence. The effect of rye cover cropping on the proportion of persistent seeds of large crabgrass was independent of tillage (Table 3.4; no tillage*cover crop interaction). Contrary to our hypothesis, large crabgrass seed persistence was consistently greater in winter rye treatments compared to the no cover crop control at all removal dates although this difference was reduced in July (Figure 3.3). For spring and summer sampling

dates, large crabgrass viability was two-fold greater in winter rye compared to no cover crop treatments.

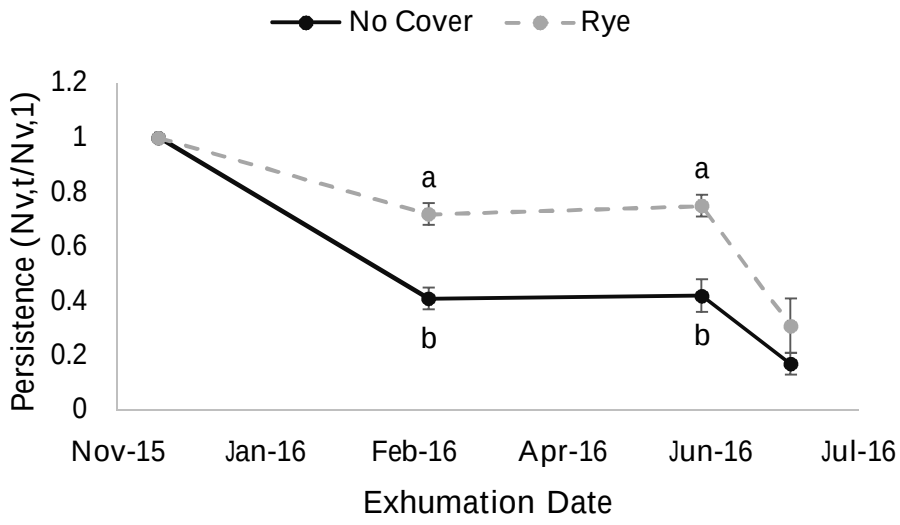

Figure 3.3: Cover crop effect on proportion persistent (mean ± SE) large crabgrass between December and 10.5 months after burial. Different letters indicate significant differences between tillage treatments. Means were separated using Fisher's Protected LSD at α=0.05.

Crabgrass seeds had greater long-term persistence regardless of tillage treatment, suggesting that winter rye residue affects viability regardless of whether the residue is left on the surface in ST or incorporated into the soil in FWT. The mechanisms responsible for this effect are unclear. Perhaps rye increased persistence by serving as an alternative food source to microbes which resulted in lower decay of crabgrass seeds. Alternatively, rye may have changed soil edaphic conditions to disfavor decay agents of crabgrass. For example, rye residue may buffer soil temperature and increase soil moisture.

Effects of fungicide treatment on persistence. After one month of burial Powell amaranth seeds that received the fungicide coating had lower proportional viability than un-treated seeds.

However, by June the seeds with fungicide treatment did not have significantly different persistence relative to viability in December (Table 3.3). For large crabgrass, fungicide treated seeds had higher proportional viability than uncoated seeds. Again, the fungicide effect did not last and the proportion of persistent crabgrass seeds in March did not differ between fungicide treatments.

Contrary to our hypothesis, the effects of tillage and cover crop on persistence did not depend on fungicide treatment for either species at either sampling date (no fungicide*tillage or fungicide*cover crop interactions; Tables 3.3 and 3.4). This lack of an interaction with either factor suggests that tillage and cover crop effects are not mediated by fungal pathogens. However, two of the three fungicides are known to have short half-lives; captan has a one to ten day half-life (Kamrin 1997) and trifloxystrobin has a two to 16 day half-life (Krieger 2001). Conversely, metalaxyl can have a much larger half-life at seven to 170 days (Kamrin 1997). In addition, captan quickly degrades in water (Kamrin 1997), trifloxystrobin forms strong bonds with soil particles (Krieger 2001) and metalaxyl is extremely soluble in water but does not bind well to soil particles (Kamrin 1997). Given how strongly environmental factors could influence individual fungicide persistence, the fungicide treatment used in this study may have only been active soon after burial and not for long enough to establish a detectable fungicide effect at the time intervals observed. Furthermore, there was not a tillage effect for either Powell amaranth or large crabgrass until eight and half months after burial and this was beyond the timeframe that fungicide coated seeds were used.

It should also be noted that germination of Powell amaranth seeds in petri-dish studies was sometimes (but not consistently) influenced by fungicide treatment. The germination of

large crabgrass seeds in petri-dishes did not show a fungicide effect on emergence. This may help explain why Powell amaranth viability declined with fungicide treatment due to an increase in fatal germination.

Experiment 2: Role of Light and Oxygen on Tillage-Mediated Seed Persistence. *Effects of light on persistence.* In FWT treatments, seeds exposed to light during the spring tillage exhumation event had a lower proportion of persistent seeds at a subsequent sampling date than seeds kept in darkness (Figures 3.4 and 3.5). After one month of burial following tillage, Powell amaranth seeds that had been exposed to light had 48% lower viability than those kept in darkness. Similarly, large crabgrass seeds exposed to light had 62% lower viability than those kept in darkness (Figure 3.5).

These results suggest that light exposure that occurred while seed bags were exhumed during tillage triggered subsequent fatal germination upon reburial, resulting in lower viability compared to both those seeds kept in darkness and those seeds left in the soil in ST treatments. Petri-dish germination trials supported this concept with crabgrass seeds showing a positive response in germination to light availability. However, the effect of light on Powell amaranth germination was less clear, with seeds tending to have higher germination in darkness. Previous studies have shown that light exposure increases germination of a crabgrass species (Tang et al. 2010) as well as species in the Amaranthus genus closely related to Powell amaranth (Gallagher and Cardina 1998; Liebman et al. 2001).

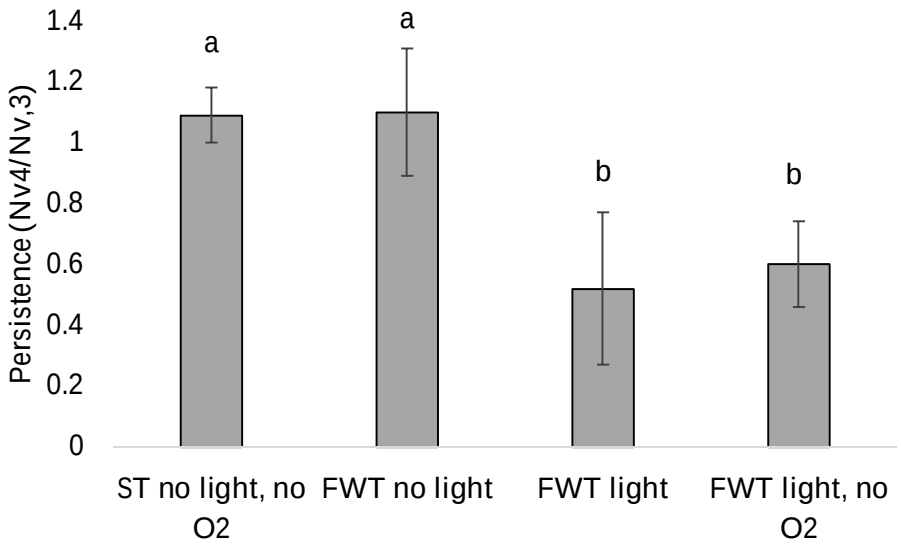

Figure 3.4: Exhumation condition effect on proportion persistent (mean ± SE) Powell amaranth seed one and a half months after tillage exhumation event. All seeds were placed within the no cover treatment. Different letters indicate significant differences between exhumation treatments within a given species. Means were separated using Fisher's Protected LSD at α=0.05.

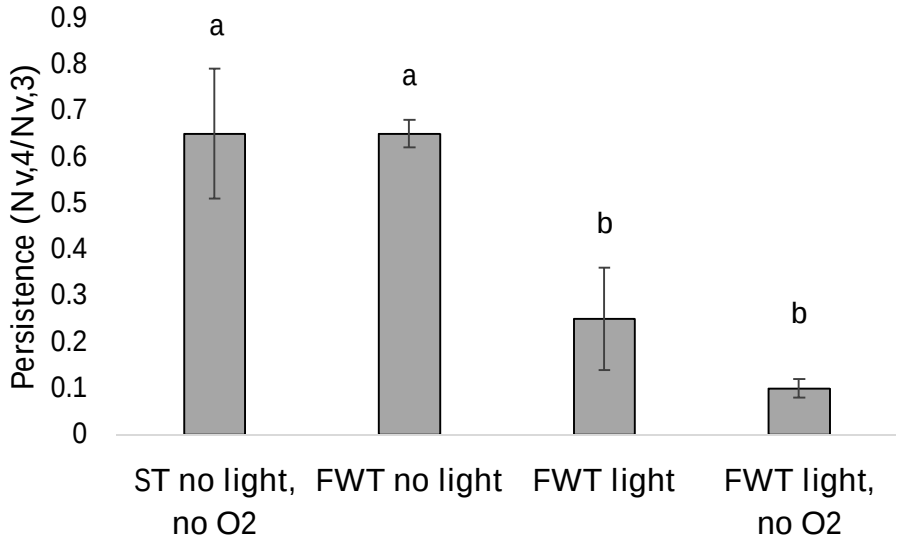

Figure 3.5: Exhumation condition effect on proportion persistent (mean ± SE) large crabgrass seed one and a half months after tillage exhumation event. All seeds were placed within the no cover treatment. Different letters indicate significant differences between exhumation treatments within a given species. Means were separated using Fisher's Protected LSD at α=0.05.

If light exposure were the only factor influencing tillage effects on seed persistence, we would expect no difference in persistence between seeds from FWT treatments exhumed and reburied in darkness, and those left in ST treatments. In our experiment, there was no difference in persistence between tillage types when we controlled for light exposure during FWT (Figures 3.4 and 3.5). These results support our hypothesis that tillage-induced light exposure was a mediating factor between tillage and seed persistence, and suggests that light exposure during tillage reduced persistence by stimulating fatal germination. While fresh seeds may not show germination responses to light there are multiple studies showing that seeds gain sensitivity to light stimulation during burial (Wesson and Wareing 1967, 1969), perhaps due to seasonal phytochrome sensitivity (Taylorson 1972). Therefore tillage-based light exposure may deserve more attention when considering the effects of tillage on seed persistence.

There are numerous other studies utilizing buried and retrieved weed seed bags in agricultural contexts. However many of those studies seemingly do not account for the effects of light exposure at the time of tillage (Gallandt et al. 2004). In addition, many studies of weed seed persistence do not control for light exposure or discuss tillage-induced light exposure when interpreting their results (Davis et al. 2005; Ullrich et al. 2011).

Effects of oxygen on persistence. Underwater treatments intended to reduce oxygen exposure of seeds during tillage exhumation did not significantly change the persistence of either Powell amaranth or large crabgrass compared to those that were exhumed under ambient O_2 conditions (Figure 3.4). This result is inconsistent with the hypothesis that tillage effects on seed persistence are mediated by changes in O_2 exposure.

Seed bags in the soil were presumably exposed to lower levels of oxygen than were present in the general atmosphere. Therefore it was expected that when seed bags were removed from the soil to accommodate spring tillage, the exposure to higher levels of oxygen would have promoted seed germination. Weed seeds within the seedbank would similarly be exposed to a period of increased oxygen exposure during a tillage event that could promote seeds in the seedbank to germinate. Ultimately this would reduce weed seed viability in the seedbank as seeds would either successfully or fatally germinate. While the data do not support this hypothesis, these results may have been influenced by faulty methods. During the spring tillage exhumation event, the seed bags were stored in sealed plastic bags submerged under water so as to prevent seed exposure to high levels of oxygen. During that time the plastic bags leaked and all seed bags became saturated with water for a period of several hours. Therefore the lack of a significant response between seed viability and reduced exposure to oxygen may have been influenced by the over-exposure to water.

Summary and Conclusions

Tillage Effects. Experiment 1 demonstrated that seeds of both weed species had lower persistence in FWT treatments, but only following exhumation and reburial that occurred during tillage in early spring. This result suggests that tillage effects may have been due to short-term effects of tillage on factors influencing persistence including exposure to light and oxygen. In addition, this experiment showed that there was not a significant interaction between fungicide treatment and tillage treatment suggesting that fungal pathogens did not mediate the effects of tillage on seed persistence. Experiment 2 provided evidence that for both species, light exposure was an important factor explaining tillage effects. When seeds were kept in darkness during the tillage operations, seed persistence in FWT was increased to levels similar to those in ST treatments.

Cover Crop Effects. Crabgrass seeds buried in rye cover crop treatments had greater long-term persistence than those in no cover crop treatments regardless of tillage, although the reasons for this effect are unclear. Rye may have resulted in shifts in edaphic conditions including soil temperature and moisture which dis-favored decay agents of crabgrass. On the other hand, rye residue may have served as an alternative food source to microbes which allowed for greater crabgrass seed survivorship. In addition, experiment 1 showed that there was not a significant interaction between fungicide treatment and cover crop treatment suggesting that fungal pathogens did not mediate the effects of cover crops on seed persistence.

In summary, our results were not consistent with the hypothesis that weed seed persistence would be lower under conservation agricultural practices. In fact, persistence of

seeds of both species was higher under ST compared with FWT and the persistence of large crabgrass was higher under rye cover cropping compared to no cover crop. Our results also did not support the hypothesis that tillage and cover crop effects on seed persistence were mediated by fungal pathogens. Rather, we found that tillage effects on persistence were explained primarily by light exposure during tillage operations. This finding is potentially of great importance when interpreting studies evaluating the impact of tillage on seed persistence.

Overall, our findings suggest that shifts in weed species density and composition under conservation agricultural practices are driven in part by differences in seed persistence. Although we found that light exposure was a major factor explaining tillage-induced changes in persistence, the mechanisms behind cover crop induced changes in persistence remain unclear. Future studies evaluating potential mechanisms responsible for the cover crop effects observed in this study would be valuable for understanding and managing large crabgrass, a major weed problem in both conventional and conservation agricultural systems.

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LITERATURE CITED

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CHAPTER FOUR: Tillage and Cover Crop Effects on Extracellular Enzymes of Soils and Seeds

Abstract

Conservation agriculture management including reduced tillage and cover crop use can alter soil microbial communities while also changing annual weed seedbank dynamics. The objectives of this study were to 1) evaluate the impacts of tillage (full-width tillage [FWT] or strip-tillage [ST]) and cover crop (none or winter rye) on microbial activity and 2) to investigate potential relationships between weed seed persistence and indicators of microbial activity. Tillage and cover crop treatments were imposed on the same plots for six years in a sweet corn-snap bean- cucurbit rotation on sandy soils in southwest Michigan. In October of 2015, seeds of Powell amaranth (*Amaranthus powellii*) and large crabgrass (*Digitaria sanguinalis*) were buried in mesh bags. Seeds were recovered and soils sampled in October (soils only) and December 2015, as well as March, June, July, and September of 2016. Soils were evaluated using assays for extracellular enzyme activity (EEA) for β-glucosidase (BG), leucine aminopeptidase (LAP), acid phosphatase (PHOS), phenol oxidase (PHEN), and peroxidase (PER). In addition, assays were modified to test individual seeds of both weed species for the same enzymes excluding acid phosphatase. Soil enzyme activity for BG, LAP, and PHOS tended to be greater under ST compared to FWT treatments while only BG had greater activity in rye cover crop compared with no cover crop treatments. Enzyme activity on intact and decayed seeds differed by species but was not affected by tillage or cover crop history with only one exception. For amaranth but not crabgrass, enzyme activity on decayed seeds was higher than that on intact seeds. Soil sample EEA results did not correlate well with either amaranth or crabgrass EEAs suggesting

that microbial activity in the soil and microbial activity on buried seeds were independent. Soil EEAs in July and September were positively related with Powell amaranth persistence while only BG activities in March, June and July were positively correlated with large crabgrass persistence. Enzyme activity and seed persistence for both Powell amaranth and large crabgrass were not well correlated.

Nomenclature. Powell amaranth, *Amaranthus powellii* S. Wats. AMAPO; large crabgrass, *Digitaria sanguinalis* (L.) Scop. DIGSA; winter rye, *Secale cereal*; hairy vetch, *Vicia villosa*.

Key Words. Strip-tillage, enzyme assay, β-glucosidase, leucine aminopeptidase, acid phosphatase, phenol oxidase, peroxidase, seed persistence.

Introduction

Microbes. Summer annual weed seeds within the seedbank may be exposed to bacteria and fungi that colonize and consume seeds as a carbon source, thereby reducing persistence through seed decay and death. Some seeds come into contact with microorganisms while still on the parent plant, resulting in seedborne microbes. For example, *Acinetobacter* spp., *Bacillus* spp., *Erwinia* spp., *Pseudomonas* spp., and *Xanthomonas* spp. have been recovered from seeds prior to seed rain (Kremer 1987). Once seeds are shed onto the soil and become incorporated into the seedbank, seeds become exposed to additional soilborne microbes including fungi such as *Rhizopus* spp., *Pythium* spp., *Alternaria* spp., and *Fusarium* spp. (Wagner and Mitschunas 2008). An intensive review of European studies indicates that saprophytic fungi in the soil are important in reducing the weed seedbank of many species (Wagner and Mitschunas 2008). Fungi in the soil can exude toxins that ultimately damage or kill seeds by preventing germination, destroying seed coats, or promoting solute leakage from cells (Halloin 1986; Harman 1983). Seedborne and soilborne fungi have had additive effects on seed persistence, with both microbial sources causing greater seed loss than either source alone (Kiewnick 1964).

Effects of Management on Microbes. Not surprisingly, agricultural management practices such as tillage and cover cropping can alter the abundance and diversity of microbes in the soil (Drijber et al. 2000). While the impact of strip tillage on fungal communities has not been closely examined there have been multiple studies on no-tillage systems. Since the between-row zones of a strip-tilled field are left un-tilled, the responses of microbial communities to no-till may be similar to those in the between-tow zone of a strip-tilled field. According to a meta-analysis by

Wardle (1995), there is compelling evidence that no-till systems have greater microbial biomass than conventional tillage systems. Recent studies across the United States are consistent with these findings that microbial abundance and/or activity are greater under no-till (Frey et al. 1999; Helgason et al. 2009; Runion et al. 2004) especially when the reduced tillage system is combined with cover crop use (Minoshima et al. 2007). Few such studies have been conducted in strip-till systems. Differences in disturbance patterns and spatial heterogeneity within strip-till can alter population dynamics of weeds compared to no-till (Brainard et al. 2013)and similar differences may occur across microbial communities.

The mechanism behind increased microbial abundance in conservation agriculture systems may be the result of increased soil moisture and decreased soil temperature. Despite original suggestions that fungal abundance in no-till systems is inversely related to soil moisture (Hendrix et al. 1986), fungal biomass has been found to be positively related to soil moisture as influenced by no-tillage and conventional-tillage operations (Frey et al. 1999); no-till soils consistently had greater water content compared to conventional-till soils regardless of a climatic gradient. However, Chen et al. (2007) found that soil moisture did not have a major effect on fungal biomass while there was a crop species effect on microbial community composition. At soil temperatures of 25-30^{0}C maximum fungal growth was observed with greater tolerance of lower temperatures than higher temperatures (Pietikäinen et al. 2005). While these results occurred in a controlled setting and not in the field they could suggest that fungi have greater seasonal growth under systems that allow for warmer soils. Bacteria have a reduced tolerance of lower temperatures (Pietikäinen et al. 2005) and therefore fungi would have a competitive advantage over bacteria in systems that retain surface residues and maintain

cooler soil temperatures. In the event that beneficial bacteria prevent pathogenic fungi from attacking seeds, these results could further indicate that cooler soil temperatures (such as seen in no-till or strip-till systems) could reduce beneficial bacteria abundance while promoting pathogenic fungi abundance.

Differences in fungal biomass and activity have also been observed across soil depth and can be influenced by tillage and soil type. Fungal biomass was greater in no-till versus conventional tillage systems at a shallow depth (typically 0-5cm) but not as consistently at deeper depths (Frey et al. 1999; Helgason et al. 2009; Lupwayi et al. 2004). However, Spedding et al. (2004) on a sandy loam/loamy sand found no such shift in fungal:bacterial abundance and no effect of either tillage (conventional moldboard plow, reduced tillage, or no-tillage) or corn crop residue on fungal phospholipid fatty acids (PLFA) alone.

Fungal community biomass typically fluctuates during the course of a year and the timing of tillage may influence what responses are seen. In a cotton cropping system, microbial community differed across tillage systems in February and May following spring tillage but not in October following fall tillage (Feng et al. 2003). There is evidence that microbial biomass and activity increase as a result of tilling residue into the soil, although this effect is short-lived (Lee et al. 1996; Lynch and Panting 1980).

Enzymes as Indicators of Microbial Activity. While many studies have used PLFA analysis (Chen et al. 2007; Feng et al. 2003), fatty acid methyl esters (FAME) analysis (Drijber et al. 2000), or DNA extraction techniques (Chee-Sanford et al. 2010) to analyze soil microbial communities, microplate techniques for extracellular enzyme activities (EEA) assays have been used less

extensively. EEA assays are a high through-put analysis successfully used to measure the functional structure of soil microbial communities. Multiple enzymes have been studied including β-1,4-glucosidase (BG), acid phosphatase (PHOS), leucine aminopeptidase (LAP), phenol oxidase (PHEN), and peroxidase (PER) (Saiya-Cork et al. 2002; Sinsabaugh et al. 2008). For the first three enzymes, BG degrades cellulose primarily by hydrolyzing cellobiose to glucose (Ljungdahl and Eriksson 1985), PHOS enzymes hydrolyze phosphomonoesters thereby releasing phosphate (Toor et al. 2003; Turner et al. 2002), and LAP hydrolyzes leucine from polypeptides and is considered to be an indicator of peptidase potential (Sinsabaugh and Foreman 2001; Stursova et al. 2006). Phenol oxidase and peroxidase are two classes of enzymes primarily responsible for degrading polyphenols such as lignin and tannin (Kirk and Farrell 1987). Phenol oxidases are specifically able to degrade phenolic groups (Mayer and Staples 2002) while peroxidases degrade aromatic compounds (Hofrichter 2002). Many of these enzymes could be important in degrading seeds into nutritional components, and while some researchers have used DNA extraction techniques to understand microbial communities on seeds (Chee-Sanford et al. 2010; Links et al. 2014), no previous studies have reported EEA results from seeds.

Objectives and Hypotheses. Improved understanding of the relationship between conservation agricultural practices, soil microbial activity, and weed seed persistence will help identify practices which improve long term crop productivity and potentially reduce weed infestations. The objectives of this study were to evaluate the impacts of strip-tillage and a winter rye cover crop on microbial activity, as measured by extracellular enzyme assays in both the soil and on

seed surfaces, and to investigate potential relationships between weed seed persistence and indicators of microbial activity. We hypothesized that 1) winter rye cover crops and strip-tillage would result in greater extracellular enzymes in the soil by providing a more suitable food source and stable habitat for microbes compared to conventional systems; 2) extracellular enzymes on seed surfaces will vary by weed species and be correlated with soil enzymes; and 3) extracellular enzymes in both seeds and soils will be negatively correlated with the persistence of weed seeds in the soil.

Materials and Methods

Long-term Trial Experimental Treatments and Design. Extracellular enzymes were monitored in conjunction with the weed seed burial experiment described in Chapter Three. The experiment was conducted at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor, Michigan (42.085244^0 N, 86.358736^0 W). This research was conducted within a subset of treatments in a long-term tillage trial that was initiated in September 2008 on Oakville fine sand. Experimental treatments included all combinations of three factors: tillage (strip-tillage [ST] vs. conventional full-width tillage using a moldboard plow [FWT]), cover crop (no cover crop [no cover], winter rye [rye], or either a hairy vetch winter rye mix (until 2014) or hairy vetch (since 2014)), and weed management (reduced input [low] vs. standard grower practice [high]). Treatments were imposed in the same plots each year with crops following a three year rotational sequence of sweet corn-snap bean-cucurbit crop (butternut squash in 2011 and pickling cucumber in 2014). Plots were arranged in a split-split plot design with tillage as the main plot factor, cover crop as the sub-plot factor, and weed management as the sub-sub plot factor. Tillage main plots measured 11.4m x 18.3m and were arranged in a randomized complete block design with four replications. Weed management split-split plots were 3.8m x 9.1m with either two (winter squash) or five rows (all other crops) per plot.

Seed burial and soil sampling were conducted only in the low weed management sub-sub plots of this long-term experiment to avoid any confounding effects of historical weed management intensity on seed persistence. In addition, only the rye and no cover crop control plots were included. Therefore, only the details of these treatments will be described.

Field Management. Winter rye cover crops were drilled at 125kg/ha in September of each year using a grain drill with 19cm between-row spacing (Table 4.1). Tillage occurred in May or early June depending on the crop. Full width tillage consisted of moldboard plowing followed by disking and field cultivating. Strip-tillage was accomplished using either a Hiniker 6000 strip-tiller (for sweet corn, snap beans, and cucumbers) or an Unverferth 120 subsoiler (for winter squash). Both strip-tillage implements were equipped with a row-cleaner (to remove cover crop residue), a shank, offset disks, and a rolling basket. Strip tillage resulted in an approximately 25cm wide by 30cm deep zone of disturbed soil into which crops were planted.

Table 4.1: Timing of relevant field operations and experimental procedures

	Date	Field Operation	Experiment Operation
2015	Aug-24	sweet corn harvested	
	Sept-4	corn residue disked	
	Sept-10	rye seeded	
	Oct-27		soil samples collected, weed seed bags buried
	Dec-1		soil samples collected, exhumation 1
2016	Mar-22		soil samples collected, exhumation 2
	May-19	rye cover terminated	
	Jun-1	tillage	soil samples collected, exhumation 3, all bags removed and reburied in conventional-till
	Jun-2	snap beans planted	
	Jun-10	overhead irrigation installed	
	Jul-27		soil samples collected, exhumation 4
	Jul-28	beans harvested	
	Aug-17	bean residue disked	
	Aug-23	rye seeded, 2 bushel/acre	
	Sept-8		soil samples collected, exhumation 5

Weed management in the low intensity treatments varied by crop, and included both herbicides and occasional mechanical cultivation (in full width tillage treatments only). In snap beans and sweet corn, herbicides included S-metolachlor (Dual Magnum, 1 pint/acre) pre-emergence with a post-emergence application of sodium salt of bentazon (Basagran, 0.75 quarts/acre) and fomesafen sodium salt (Reflex, 0.5 pint/acre). In some years, snap beans also received a post-emergence application of clethodim (SelectMax, 1 pint/acre) to control grass weeds as needed. For cucurbit crops, herbicides included a pre-emergence application of ethalfuralin/clomazone (Strategy, 3 pints/acre) and a post-emergence application of clethodim (SelectMax, 1 pint/acre). In FWT treatments, cultivation with s-tine sweeps was also used as needed to manage weed escapes between crop rows. Rates and timings of herbicide applications differed slightly by year and crop but were identical for all treatments within a given year.

Extracellular Enzyme Assays on Seeds. *Weed seed burial experiment and processing.* A seed burial experiment was conducted to evaluate the effects of tillage (ST vs FWT) and cover crop (no cover vs. winter rye) on seed persistence of Powell amaranth (*Amaranthus powellii* S. Wats., AMAPO, collected in 2011 from Hickory Corners, MI) and large crabgrass (*Digitaria sanguinalis* (L.) Scop., DIGSA, collected in 2012 from Benton Harbor, MI). In October of 2015, seeds were placed with white silica sand in mesh bags and buried at a depth of 10.2cm in all four cover crop x tillage treatments (FWT+no cover, FWT+ rye, ST+no cover, FWT+rye). Sufficient bags were buried for removal at multiple subsequent exhumation dates at which time seed viability of each species were evaluated. Intact and decayed seeds of both DIGSA and AMAPO were

retrieved in July of 2016 and frozen at -80^{0}C for extracellular enzyme assays. Complete details of the seed burial experiment can be found in Chapter Three.

Assay procedures. Recovered seeds were placed in 96-well microplates with one seed per well and four replicate wells per sample. Leucine aminopeptidase and β-glucosidase assays were fluorimetric. Each sample well received 25µL of 200µM substrate solution (Table 4.2) with 100µL distilled water. Control wells contained one seed and 125µL distilled water. Negative control wells contained 25µL substrate solution with 100µL distilled water and quench standard wells for both species contained 25µL of substrate specific standard (Table 4.2), 125µL distilled water, and one seed. Reference standard wells contained either 50µL, 25µL, or 10µL of standard with 75µL, 100µL, or 115µL distilled water, respectively. Negative control, quench standard, and reference standards all utilized eight replicate wells. Phenol oxidase and peroxidase assays were colorimetric. Each sample well received either 25µL of L-DOPA with 125µL distilled water. Control wells contained one seed and 125µL distilled water. Each peroxidase test and control well also received 10µL of 0.3% H_2O_2. Negative control wells contained 100µL distilled water, 25µL L-DOPA substrate and 10µL H_2O_2 (peroxidase only). Blank standard wells contained 125µL distilled water, one seed, and 10µL H_2O_2 (peroxidase only). Negative control and blank standards all utilized eight replicate wells. All microplates were lightly hand-agitated and then incubated at 25^{0}C for 18hr at which time all fluorimetric reactions were terminated using 10µL of 1.0M NaOH per well. Plates were analyzed on a Synergy H1 plate reader (BioTek, Winooski, VT, USA) where fluorescence was measured with 355nm excitation and 450nm emission filters and colorimetric assay absorbance was measured at 460 nm.

Table 4.2: Extracellular enzymes assayed with their abbreviations, substrates, and standards

Enzyme	Abbreviations	Substrate	Standard
β-1,4-glucosidase	BG	4-methylumbelliferyl-β-D-glucopyranoside	4-methylumbelliferone (MUB)
Acid phosphatase	PHOS	4-methylumbelliferyl-phosphate	4-methylumbelliferone (MUB)
Leucine aminopeptidase	LAP	L-leucine-7-amino-4-methylcoumarin hydrochloride	7-amino-4-methylcoumarin (MC)
Phenol oxidase	PHEN	3,4-dihydroxy-L-phenylalanine (L-DOPA)	
Peroxidase	PER	3,4-dihydroxy-L-phenylalanine (L-DOPA)	

Extracellular Enzyme Assays on Soils. *Soil sampling and processing.* At the time of seed bag burial and at each exhumation, soil samples were collected by taking eight 2.6cm diameter cores to a 12.7cm depth from areas adjacent to each burial site. Soils were kept chilled at 4^0C before being passed through a 4mm sieve to remove residue and debris. Small subsamples were then placed in -80^0C storage prior to enzyme assay analysis.

Assay procedures. Soil suspensions were prepared by homogenizing one gram of soil with 125mL distilled water for 30s. Suspensions were stirred on a magnetic stir plate to promote sample homogeneity as 200µL aliquots were pipetted in 96-well microplates. Each soil sample had eight replicate wells used to determine a quench coefficient and 16 replicate test wells per enzyme assayed. Leucine aminopeptidase, acid phosphatase, and β-glucosidase assays used substrates fluorimetrically labelled with either 4-methylumbelliferone (MUB) or 7-hydroxy-4-methylcoumarin (MC). To each soil sample well either 50µL fluorescent standard (quench wells) or 200µM substrate solution (assay wells; Table 4.2). Negative control wells contained 50µL of MUB or MC substrate solution with 200µL distilled water (Table 4.2). Reference standard wells contained either 50µL, 25µL, or 10µL of MUB or MC standard with 200µL, 225µL, or 240µL distilled water, respectively. Negative control, quench standard, and reference standards all utilized eight replicate wells. Phenol oxidase and peroxidase assays were colorimetric. Each sample well received either 50µL of L-DOPA or 50µL distilled water for control. Each peroxidase test and control well also received 10µL of 0.3% H_2O_2. Negative control wells contained 200µL distilled water, 50µL L-DOPA substrate and 10µL H_2O_2 (peroxidase only). Blank standard wells contained 50µL distilled water, 200µL soil sample suspension, and 10µL H_2O_2 (peroxidase only). Negative control and blank standards all utilized eight replicate

wells. All microplates were incubated at 25^{0}C for 18hr at which time all fluorimetric reactions were terminated using 10µL of 1.0M NaOH per well. Plates were analyzed on a Synergy H1 plate reader (BioTek, Winooski, VT, USA) where fluorescence was measured with 355nm excitation and 450nm emission filters and colorimetric assay absorbance was measured at 460 nm.

Statistical Analysis. Enzyme activities for BG, PHOS, and LAP in soil samples were calculated as nmols of substrate converted per gram of soil per hour according to the following series of equations:

(1) Emission coefficient: Ce = slope of standards concentration vs. fluorescence

(2) Quench coefficient: Cq = (avg. fluor. soil slurry + standard)/(buffer + standard)

(3) Net fluorescence: Fnet = (Fluorescence/Cq)-(negative control)-(sample blank)

(4) Activity: (nmols/h/g) = ((Fnet-Ce)/Ce*Vs)/(Va*t*Ms)

where Vs is the volume of soil suspension, Va is the assay volume per well, t is incubation time, and Ms is the mass of dry soil. Enzyme activities for PHEN and PER in soil samples were calculated as nmols of substrate converted per gram of soil per hour according to the following equation:

(5) Activity: (nmols/h/g) = (absorbance-negative control)*Vs)/(Cex*Va*t*Ms)*1000

where the extinction coefficient (Cex) is 7.9 μmol^{-1} for oxidase assays. Enzyme activities for BG and LAP on recovered seeds were calculated as nmols of substrate converted per hour per seed according to the following series of equations:

(6) Emission coefficient: Ce = slope of standards concentration vs. fluorescence

(7) Net fluorescence: Fnet = (Fluorescence)-(negative control)-(sample blank)

(8) Activity: (nmols/h) = (Fnet-Ce)/Ce/t/Ns

where Ns is number of seeds. Enzyme activities for PHEN and PER on recovered seeds were calculated as nmols of substrate converted per hour according to the following equation:

(9) Activity: (nmols/h) = (absorbance-negative control)/(Cex*t*Ns)*1000

where the extinction coefficient (Cex) is 7.9 μmol^{-1} for oxidase assays.

For all six soil sampling dates, the effects of tillage and cover crop treatments on BG, PHOS, LAP, PHEN, and PER were analyzed separately using the PROC GLIMMIX procedure in Statistical Analysis System 9.4 (SAS Institute Inc. 2002-2012. Cary, NC). At these sampling

dates, data were analyzed as a split-plot design with tillage and cover crop treated as fixed effects, and replicate, and replicate x tillage as random effects. The effects of tillage on intact seed EEA were analyzed separately for AMAPO and DIGSA using PROC GLIMMIX. The effects of tillage and cover crop treatments on decayed seed EEA were also analyzed separately by weed species using a split-plot design in PROC GLIMMIX. Tillage and cover crop were treated as fixed effects and replicate and replicate x tillage as random effects. Additional analyses with either the weed species (AMAPO vs. DIGSA) or seed condition (intact vs. decayed) were conducted in PROC GLIMMIX. Where main or interactive effects were significant, treatment mean separation occurred using Fisher's Protected LSD at α=0.05. Correlation analyses between soil EEA and seed EEA, soil EEA and seed persistence, and seed EEA and seed persistence were all conducted separately using the PROC CORR procedure of SAS.

Results and Discussion

Tillage and Cover Crop Effects on Soil Extracellular Enzymes. Tillage affected soil extracellular enzymes for BG, LAP (all but October and March), and PHOS (all but March) with greater quantities of enzymes found in ST treatments (Tables 4.3, 4.4, and 4.5; Figure 4.1). A rye cover crop increased BG at the December and June sampling dates and in March the ST+rye treatment had larger quantities of BG compared to all other treatments (Table 4.3; Figure 4.2). PHEN and PER activities were not influenced by tillage or cover crop (Tables 4.6 and 4.7; Figures 4.1 and 4.2). These results support our hypothesis that a winter rye cover crop and ST would have greater extracellular enzyme activity and is in agreement with similar findings of greater microbial activity or biomass in no-tillage compared with conventional tillage systems (Frey et al. 1999; Helgason et al. 2009; Runion et al. 2004) and in cover cropped versus no cover crop systems (Mendes et al. 1999; Minoshima et al. 2007).

Soil moisture is an important driving factor of microbial activity and therefore the greater microbial activity seen under ST and a winter rye cover crop could be in response to the increased soil moisture of those treatments (see Chapter Two), similar to tillage-based soil moisture results seen by Frey et al. (1999). In addition, ST and rye treatments also tend to have greater nitrogen mineralization, which is consistent with the greater LAP activity of ST treatments in this study (Table 4.4). Increased microbial activity in ST and rye cover crop treatments may also reflect accumulation of greater soil organic matter in those treatments over the seven year period that treatments were imposed (Powlson et al. 1987).

Table 4.3: Main effects, interactions, and overall ANOVA for β-glucosidase

	OCT-2015 burial	DEC-2015 1 month	MAR-2016 4.5 months	JUNE-2016 7 months	JULY-2016 8.5 months	SEPT-2016 10.5 months
	nmol/h/g					
Tillage Main Effect						
FWT	109.22 b	120.30 b	121.91 b	156.40 b	139.34 b	130.09 b
ST	139.57 a	160.03 a	151.68 a	207.90 a	217.67 a	190.58 a
Cover Crop Main Effect						
No cover crop	116.40	131.10 b	126.28 b	158.93 b	167.88	145.00
Rye	132.39	149.22 a	147.31 a	205.38 a	189.13	175.67
Tillage x Cover Crop Interaction						
FWT						
No cover crop	-	-	118.69 b	-	-	-
Rye	-	-	125.13 b	-	-	-
ST						
No cover crop	-	-	133.87 b	-	-	-
Rye	-	-	169.48 a	-	-	-
ANOVA						
Tillage	*	**	*	*	*	*
Cover Crop	N.S.	*	**	**	N.S.	+
Tillage x Cover Crop	N.S.	N.S.	*	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.) and means followed by different letters are significantly different at α =0.05.*

Table 4.4: Main effects and overall ANOVA for leucine aminopeptidase

	OCT-2015 burial	DEC-2015 1 month	MAR-2016 4.5 months	JUNE-2016 7 months	JULY-2016 8.5 months	SEPT-2016 10.5 months
	nmol/h/g					
Tillage Main Effect						
FWT	26.77	30.34 b	33.34	22.89 b	17.15 b	15.75 b
ST	46.24	53.16 a	52.33	36.25 a	38.17 a	30.20 a
Cover Crop Main Effect						
No cover crop	33.05	38.66	34.94	27.54	25.45	21.00
Rye	39.95	44.85	50.73	31.60	29.81	23.95
ANOVA						
Tillage	+	*	N.S.	*	**	**
Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tillage x Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.) and means followed by different letters are significantly different at α =0.05.*

Table 4.5: Main effects and overall ANOVA for acid phosphatase

	OCT-2015 burial	DEC-2015 1 month	MAR-2016 4.5 months	JUNE-2016 7 months	JULY-2016 8.5 months	SEPT-2016 10.5 months
	nmol/h/g					
Tillage Main Effect						
FWT	69.33 b	82.69 b	91.29	115.99 b	97.77 b	98.84 b
ST	106.07 a	125.03 a	117.34	160.86 a	169.97 a	153.35 a
Cover Crop Main Effect						
No cover crop	83.16	97.49	93.69	125.68	122.25	117.95
Rye	92.24	110.23	114.94	151.17	145.49	134.25
ANOVA						
Tillage	*	**	N.S.	*	*	*
Cover Crop	N.S.	+	+	+	N.S.	N.S.
Tillage x Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.) and means followed by different letters are significantly different at α =0.05.*

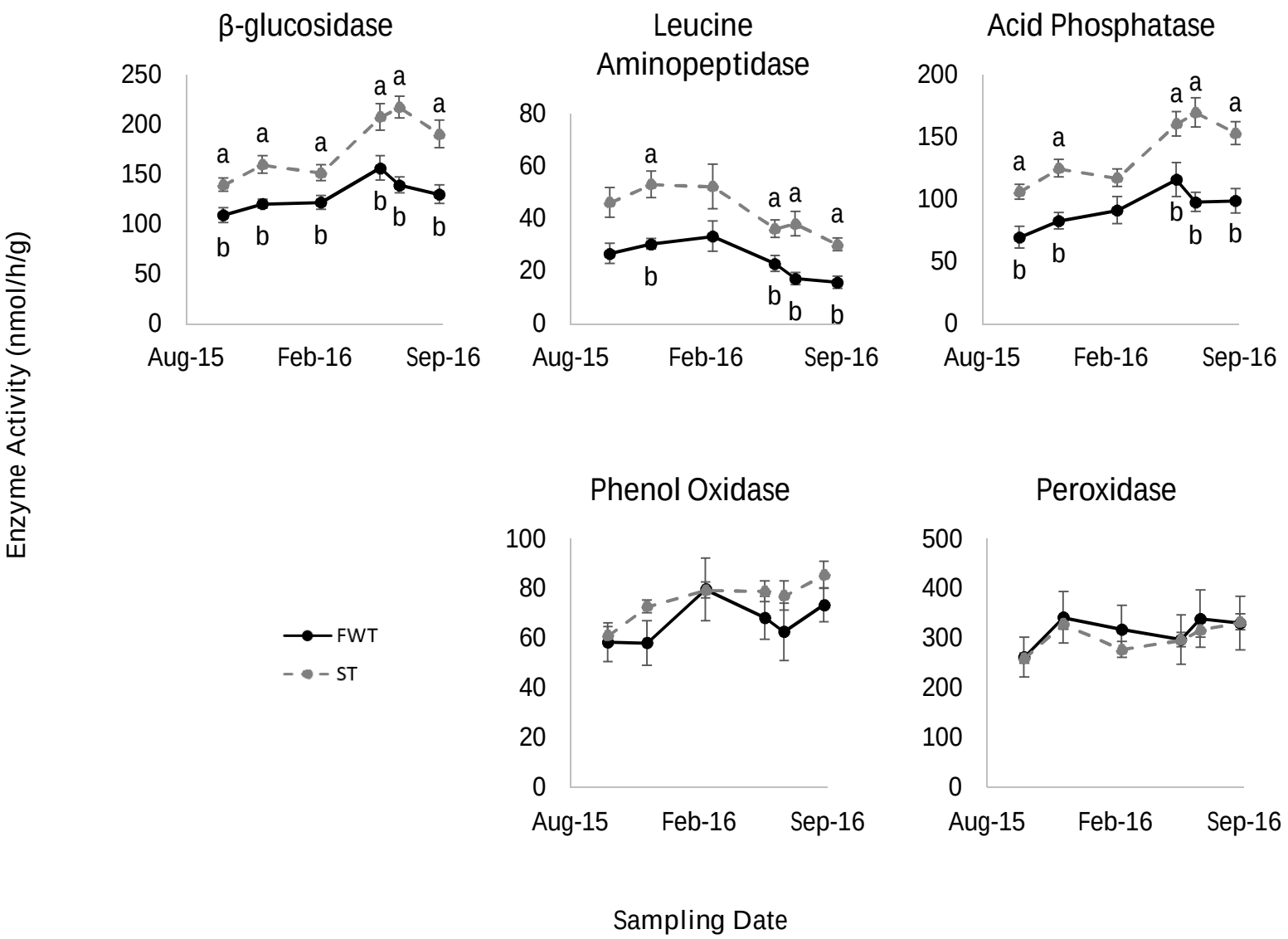

Figure 4.1 Tillage effect on soil extracellular enzyme activities. Different letters indicate significant differences between tillage treatments at α=0.05.

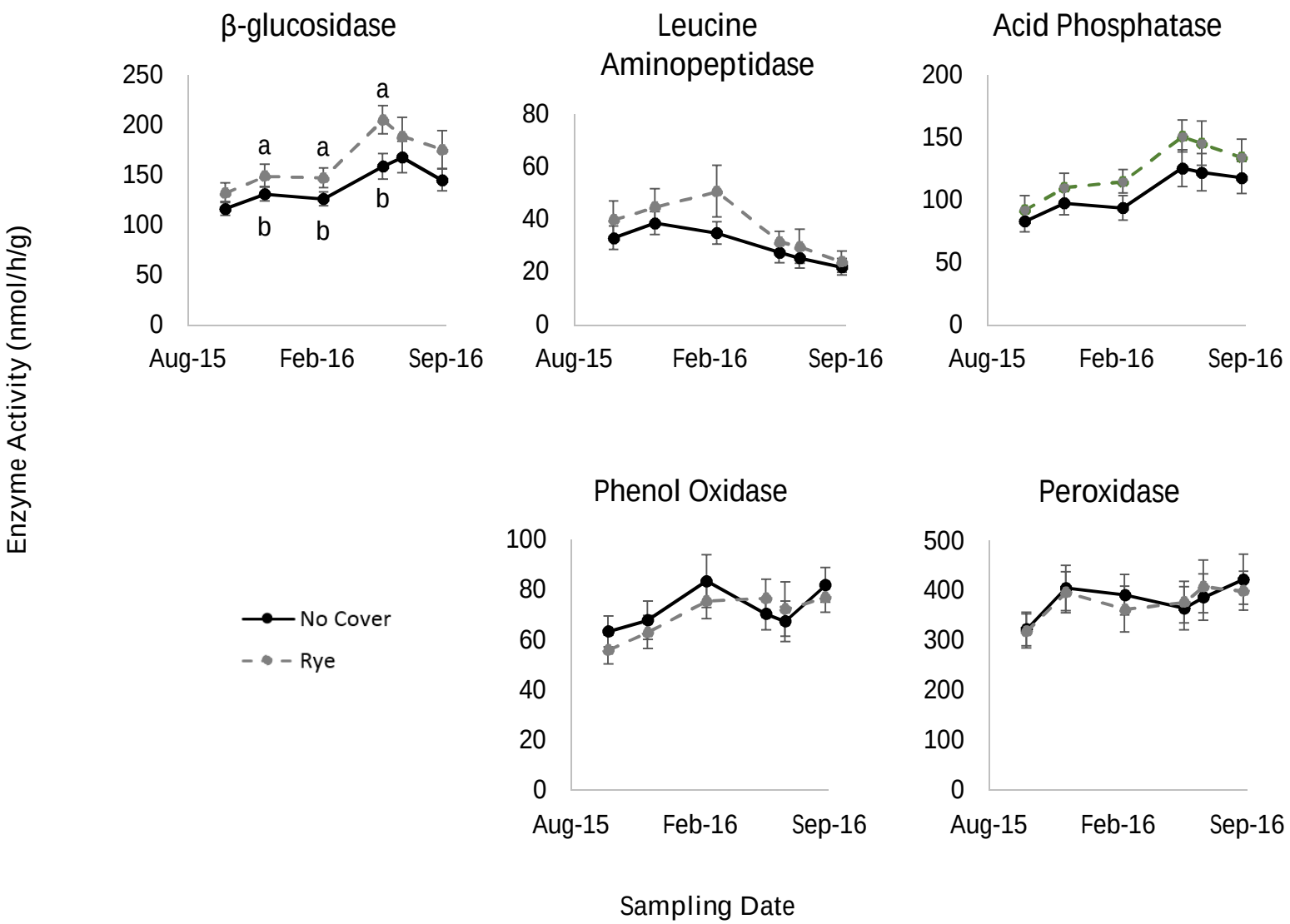

Figure 4.2 Cover crop effect on soil extracellular enzyme activities. Different letters indicate significant differences between cover crop treatments at α=0.05.

Table 4.6: Main effects and overall ANOVA for phenol oxidase

	OCT-2015 burial	DEC-2015 1 month	MAR-2016 4.5 months	JUNE-2016 7 months	JULY-2016 8.5 months	SEPT-2016 10.5 months
	nmol/h/g					
Tillage Main Effect						
FWT	58.43	58.13	79.66	68.26	62.58	73.37
ST	61.09	72.82	79.43	78.93	77.18	85.61
Cover Crop Main Effect						
No cover crop	63.41	67.84	83.46	70.47	67.47	81.93
Rye	56.11	63.11	75.62	76.72	72.29	77.06
ANOVA						
Tillage	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tillage x Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.) and means followed by different letters are significantly different at α =0.05.*

Table 4.7: Main effects and overall ANOVA for peroxidase

	OCT-2015 burial	DEC-2015 1 month	MAR-2016 4.5 months	JUNE-2016 7 months	JULY-2016 8.5 months	SEPT-2016 10.5 months
	nmol/h/g					
Tillage Main Effect						
FWT	261.97	341.95	317.92	297.48	339.15	330.61
ST	260.02	329.20	277.30	297.21	316.66	332.62
Cover Crop Main Effect						
No cover crop	259.19	337.38	307.92	294.32	319.43	340.49
Rye	262.80	333.76	287.30	300.37	336.38	322.74
ANOVA						
Tillage	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Tillage x Cover Crop	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001, or not significant (N.S.) and means followed by different letters are significantly different at α =0.05.*

Tillage and Cover Crop Effects on Seed Extracellular Enzymes. Activities for all four tested enzymes were consistently and significantly higher on decayed compared with intact amaranth seeds (Table 4.8). Enzyme activity on large crabgrass seeds did not show a clear differentiation between decayed and intact seeds: intact seeds had greater BG activity but lower PHEN activity than decayed seeds and no difference in LAP or PER enzymes were associated with seed condition (Table 4.8).

Tillage and cover crops did not affect EEA for intact or decayed seeds of either species (Table 4.9). In comparing the EEA for intact seeds of both species, Powell amaranth had significantly lower BG, LAP, and PHEN activity compared to large crabgrass (Table 4.9). Lower enzyme activity on Powell amaranth seeds compared to large crabgrass may have been due to inherent species differences or may simply reflect differences in their seed surface areas. Preliminary measurements of seed surface areas show that large crabgrass seeds are slightly more than two times larger than Powell amaranth seeds and thus may be able to harbor larger numbers of microbes. If the differences seen here are due to species differences this would be consistent with the Kremer (1987) finding that different seed-borne bacterial groups associate with different species. Conversely, if the differences seen here are driven by differences in seed surface area this would be consistent with Links et al. (2014) who found that wheat and canola seeds had similar microbial community loads and that various plant genera share a core set of seed-associated microbes.

Table 4.8: Main effects, interactions, and overall ANOVA for intact vs. decayed seeds of both Powell amaranth and large crabgrass

	Powell Amaranth				Large Crabgrass			
	BG	LAP	PHEN	PER	BG	LAP	PHEN	PER
	nmol/h							
Tillage Main Effect								
FWT	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
Seed Main Effect								
Intact	0.0380	0.0021 b	0.0171	0.0110 b	0.1941 a	-	0.2266 b	-
Decayed	0.0140	0.0065 a	0.0665	0.0418 a	0.1241 b	-	0.2920 a	-
Tillage x Seed Interaction								
FWT								
Intact	-	-	0.0285 b	-	-	-	-	-
Decayed	-	-	0.0650 a	-	-	-	-	-
ST								
Intact	-	-	0.0057 c	-	-	-	-	-
Decayed	-	-	0.0680 a	-	-	-	-	-
ANOVA								
Tillage	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Seed	+	**	***	*	***	N.S.	**	N.S.
Tillage x Seed	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Table 4.9: Main effects and overall ANOVA for intact and decayed seeds of Powell amaranth vs. large crabgrass

	Intact Seeds				Decayed Seeds			
	BG	LAP	PHEN	PER	BG	LAP	PHEN	PER
	nmol/h							
Tillage Main Effect								
FWT	-	-	-	-	-	-	-	-
ST	-	-	-	-	-	-	-	-
Weed Main Effect								
AMAPO	0.0000 b	0.0025 b	0.0171 b	-	0.0156 b	0.0076 b	0.0734 b	-
DIGSA	0.0014 a	0.0875 a	0.2266 a	-	0.1199 a	0.0883 a	0.2853 a	-
ANOVA								
Tillage	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Weed	***	***	***	N.S.	***	***	***	N.S.
Tillage x Weed	N.S.	N.S.	N.S.	N.S.	N.S.	+	N.S.	N.S.

*Where the above P-values are statistically significant at the following α: + <0.10, *<0.05, **<0.01, ***<0.001*

Relationship between Seed and Soil Extracellular Enzymes. The correlation between enzyme activities on intact seeds and enzyme activities of soils were generally not significant (Table 4.10). For large crabgrass there were no significant correlations between enzyme activities on intact seeds recovered in July as compared to soil enzyme activity from either June or July sampling dates for BG, PHEN, or PER (Table 4.10). Powell amaranth only showed a moderately significant positive correlation for PER between activity in July soils and intact seeds (r^2=0.6685, p=0.0700; Table 4.10). The correlation between enzyme activities on decayed seeds and soils were not significant for Powell amaranth. However, for large crabgrass there was a significant positive correlation between LAP activity on decayed seeds and June soils (r^2=0.5497, p=0.0274) as well as July soils (r^2=0.7388, p=0.0011; Table 4.10). These results suggest that, while the activities of nitrogen and recalcitrant carbon acquisition enzymes (LAP and PER) are correlated throughout the soil and on buried seeds, other extracellular enzymes found in the soil are not uniformly indicative of enzymes found immediately surrounding buried seeds in the seedbank. This does not support our hypothesis that extracellular enzymes on seed surfaces would be correlated with soil enzymes.

Chee-Sanford et al. (2006) summarized the work of many researchers and concluded that soils are comprised of many microhabitats that are influenced by physicochemical gradients that could cause spatial heterogeneity in soil microorganisms. That we did not see a strong correlation between microbial activity in the soil and microbial activity on recovered seeds is supportive of this concept that microorganisms in the soil are spatially heterogeneous.

Table 4.10: Pearson's correlation coefficients between soil enzyme activities and seed enzyme activities for intact and decayed seeds of both Powell amaranth and large crabgrass

		Intact Seeds											
		Powell Amaranth					Large Crabgrass						
Soil Sample		BG	LAP	PHEN		PER	BG	LAP	PHEN		PER		
June-16	BG	-0.3673	-0.2938	-0.6875	+	0.1446	0.0233	-0.2826	-0.0909		0.1795		
	LAP	-0.2340	-0.2713	-0.6419	+	0.1626	-0.3143	0.0210	0.1449		0.2784		
	PHEN	-0.3004	0.4996	-0.2652		0.3754	-0.0646	-0.6168	0.3081		0.0927		
	PER	-0.3912	0.5634	0.1433		0.5983	-0.1208	-0.6617	+	-0.1601		0.2329	
July-16	BG	-0.1957	-0.4127	-0.8697	**	-0.1020	0.0637	-0.1444	-0.0687		-0.0078		
	LAP	-0.1138	-0.2421	-0.6490	+	-0.0539	0.0546	0.1090	-0.0181		0.1831		
	PHEN	-0.5035	0.3498	-0.3556		0.3808	-0.1078	-0.3745	0.1442		0.1731		
	PER	-0.4155	0.5131	0.2121		0.6685	+	-0.2716	-0.6003	-0.1236		0.3020	

		Decayed Seeds											
		Powell Amaranth					Large Crabgrass						
Soil Sample		BG	LAP	PHEN		PER	BG	LAP	PHEN		PER		
June-16	BG	-0.2070	-0.0032	-0.2953		0.3328	-0.1677	0.2805	0.2724		0.4947	+	
	LAP	0.0485	0.2414	-0.3037		0.2325	-0.3452	0.5497	0.4406	+	0.4579	+	
	PHEN	-0.1989	0.2662	-0.0895		0.1875	-0.3021	-0.0733	0.2009		0.1969		
	PER	-0.3690	0.1171	-0.1325		0.3497	-0.3219	-0.4742	0.0137		-0.0290		
July-16	BG	0.0736	-0.1050	-0.1085		0.3512	-0.0226	0.4788	0.3043		0.4853	+	
	LAP	0.3749	0.1301	-0.0415		0.3301	0.0713	0.7388	0.2260		0.1501		
	PHEN	-0.2000	0.4581	+	-0.2203		0.3580	-0.3711	0.0005		0.0910		0.0082
	PER	-0.4942	+	0.1288	-0.1746		0.2403	-0.4288	-0.5131	-0.0381		0.0142	

*Where the above R^2 values are statistically significant at the following α: +<0.10, *<0.05, **<0.01, and ***<0.001.*

Relationship between Soil Extracellular Enzymes and Seed Persistence. Soil EEA for BG, LAP, and PHOS were positively correlated with large crabgrass persistence in July (Table 4.11). Soil EEA in June for BG, LAP, and PHOS also showed moderate positive correlations to Powell amaranth persistence (Table 4.11). However, it is important to note that such positive correlations do not imply any direct causal relationship. Rather, they likely reflect the fact that ST increased both soil enzyme activities (Tables 4.3-4.7) and seed persistence (Tables 3.3 and 3.4), but for different and independent reasons. Higher enzyme activities under ST likely reflect long-term changes in soils typical of reduced tillage systems. In contrast, higher seed persistence under ST likely reflects reduced short-term exposure to light as discussed in Chapter Three.

Of greater interest is the positive correlation between BG activity in soils and large crabgrass seed persistence prior to tillage-induced light exposure (March-16; Table 4.11). In this case, light-induced stimulation of fatal germination had not yet occurred under FWT so a positive correlation between persistence and soil enzyme activity cannot be readily explained. The observed positive correlation between BG activity and large crabgrass persistence in March 2016 contradicts our initial hypothesis and suggests that microbes related to BG activity may be beneficial rather than detrimental to seed persistence. Beneficial microbes may help to protect seeds by producing antimicrobial compounds that prevent against attacks from harmful microbes, as speculated by Chee-Sanford et al. (2006).

Relationship between Seed Extracellular Enzymes and Persistence. Extracellular enzyme activities on recovered intact Powell amaranth seeds showed a moderate negative correlation

between PHEN and amaranth persistence at the time of seed sampling in July (r^2= -0.7137, p=0.0468) with a stronger negative correlation to seed persistence in September (r^2= -0.8842, p=0.0035; Table 4.12). Extracellular enzyme activities on recovered large crabgrass seeds showed only one significant correlation between PER activity on decayed seeds and seed persistence in July (r^2= 0.5029, p=0.0471; Table 4.12).

While the majority of correlations between seed EEA and seed persistence were not significant, two of the three significant correlations were negative. This supports our original hypothesis that extracellular enzymes on seeds would be negatively correlated with the persistence of weed seeds in the soil.

Table 4.11: Pearson's correlation coefficients for soil EEA and seed persistence

		Large Crabgrass Persistence			Powell Amaranth Persistence		
		Mar-16	Jun-16	Jul-16	Jun-16	Jul-16	Sep-16
Mar-16	BG	0.4326 +	0.3257	0.8437 ***	n.a.	n.a.	n.a.
	LAP	0.2708	0.1596	0.5270 *	n.a.	n.a.	n.a.
	PHOS	0.3139	0.1792	0.5892 *	n.a.	n.a.	n.a.
	PHEN	-0.0122	-0.0903	0.2504	n.a.	n.a.	n.a.
	PER	-0.1621	-0.0253	0.1045	n.a.	n.a.	n.a.
Jun-16	BG	0.6718 **	0.5132 *	0.8835 ***	0.1837	0.4578 +	0.5881 *
	LAP	0.2914	0.1539	0.6794 **	0.0380	0.5159 *	0.4511 +
	PHOS	0.4040	0.3132	0.7219 **	0.0963	0.4637 +	0.4661 +
	PHEN	0.2032	0.1616	0.5565 *	0.1399	0.1371	0.2221
	PER	0.0437	0.0654	0.3013	0.2190	-0.0575	-0.0549

*Where the above R^2 values are statistically significant at the following α: +<0.10, *<0.05, **<0.01, and ***<0.001.*

Table 4.12: Pearson's correlation coefficients for seed EEA and seed persistence

	Seed Persistence					
	Powell Amaranth				Large Crabgrass	
	Jul-16		Sep-16		Jul-16	
Intact Seed EEA						
BG	-0.3700		-0.4476		0.0939	
LAP	-0.6626	+	-0.5621		-0.2962	
PHEN	-0.7137	*	-0.8842	**	-0.1713	
PER	-0.3449		-0.3834		0.2280	
Decayed Seed EEA						
BG	0.1398		-0.0420		-0.3717	
LAP	-0.2369		-0.1291		0.1901	
PHEN	0.2516		0.3783		-0.0086	
PER	0.2260		0.2431		0.5029	*

*Where the above R^2 values are statistically significant at the following α: +<0.10, *<0.05, **<0.01, and ***<0.001.*

Summary and Conclusions

In summary we had hypothesized that winter rye cover crops and strip-tillage would result in greater extracellular enzymes in the soil by providing a more suitable food source and stable habitat for microbes compared to conventional systems. Results from this study did show greater activity of BG, LAP, and PHOS under strip-tillage as well as the greater activity under a winter rye cover for BG and PHOS. Specifically we saw greater activity of enzymes associated with labile carbon, nitrogen, and organic phosphorous acquisition. These elevated activity levels under ST and a rye cover were temporally consistent which is different than other studies finding short-lived effects immediately following tillage (Lee et al. 1996; Lynch and Panting 1980).

We had also hypothesized that extracellular enzymes on seed surfaces would vary by weed species and be correlated with soil enzymes. We found that enzyme activity differed between Powell amaranth and large crabgrass, especially on decayed seeds. Enzyme activities on large crabgrass seeds were often more than three times greater than activities on Powell amaranth seeds. This may have been due to inherent species differences or simply due to differences in seed surface area.

In general, we found few positive correlations between extracellular enzymes on seeds and those on the soil in which those seeds were buried. We had hypothesized that extracellular enzymes in both seeds and soils would be negatively correlated with the persistence of weed seeds in the soil. However, correlation analysis showed that the majority of significant relationships between soil enzyme activity and seed persistence were positive. Although caution must be exercised in inferring causation from such correlations, this result raises the

question of whether elevated levels of certain soil enzymes reflect changes in soil microbial communities that are beneficial rather than detrimental to weed seed persistence. Finally, for Powell amaranth, decayed seeds had higher levels of certain enzymes than intact seeds and levels of those same enzymes on seeds were generally negatively correlated with seed persistence.

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CHAPTER FIVE: Overall Conclusions and Implications for Future Work

Tillage and Cover Crop Effects on Summer Annual Emergence. One of the primary objectives of our research was to evaluate how tillage and cover crops influence the emergence of summer annual weeds and whether these effects are mediated by fungal pathogens and herbicide efficacy. In Chapter Two, we had hypothesized that, in the absence of herbicides, sown weed emergence would be lower in ST compared to FWT and lower in cover crop compared to no cover crop treatments. We found in 2015 that in the absence of herbicides there was lower weed emergence in ST+rye for both Powell amaranth and large crabgrass, supporting our hypothesis. In the second year, amaranth had lower emergence in ST while crabgrass emergence was lower in cover crop plots. These results are consistent with other studies that have shown lower Powell amaranth emergence under no-till compared with conventional tillage (e.g. Peachey et al. 2004).

We found little support for the hypothesis that the suppressive effects of strip tillage and cover cropping were mediated by fungal pathogens. However, Powell amaranth emergence in 2016 was suppressed by ST only when seeds were unprotected by fungicide treatment. Although this effect was only marginally significant it suggests that fungal pathogens may strongly influence tillage effects on emergence in some cases. This is perhaps not surprising given that tillage influenced soil moisture and nitrogen dynamics, both of which can impact the abundance of fungal pathogens like *Pythium* (Frey et al. 1999) which have known negative effects on weed emergence.

Other factors possibly mediating tillage and cover crop effects included soil temperature, soil moisture, and soil inorganic nitrogen. Soils from ST and winter rye cover crop plots tended to have cooler temperatures, lower levels of ammonium-nitrogen, and greater moisture content. These treatments also had lower sown seed emergence, which is not surprising given that Powell amaranth emergence increases as temperatures increase (Weaver et al. 1988) and at greater nitrogen availability. However, given that Powell amaranth emergence has been seen to increase as soil moisture increases (Weaver et al. 1988), it is surprising that we saw lower emergence where soil moisture was higher. This may indicate that soil temperature and the available nitrogen had a greater influence than moisture during these trials, or that higher soil moisture affected Powell amaranth emergence indirectly through increases in fungal mediated post-germination mortality.

We had also hypothesized that the efficacy of S-metolachlor might be reduced under ST treatments with cover crops, resulting in higher emergence of species sensitive to this herbicide. This hypothesis was partly supported by our finding that the efficacy of S-metolachlor on both common lambsquarters and Powell amaranth was sometimes reduced in conservation agricultural systems. However, the practical implications of this finding are unclear; even when herbicide efficacy was reduced, cover cropping and reduced tillage resulted in equivalent or lower net emergence.

Tillage and Cover Crop Effects on Summer Annual Seed Persistence. In experiments described in Chapter Three we demonstrated that seeds of both weed species had lower persistence in FWT treatments, but only following exhumation and reburial that occurred

during tillage in early spring. This result suggests that tillage effects may have been due to short-term effects of tillage on factors influencing persistence including exposure to light and oxygen. In addition, this experiment showed that there was not a significant interaction between fungicide treatment and tillage treatment suggesting that fungal pathogens did not mediate the effects of tillage on seed persistence. However, we found that for both species, light exposure was an important factor explaining tillage effects. When seeds were kept in darkness during the tillage operations, seed persistence in FWT was increased to levels similar to those in ST treatments.

Crabgrass seeds buried in rye cover crop treatments had greater long-term persistence than those in no cover crop treatments regardless of tillage, although the reasons for this effect are unclear. Rye may have resulted in shifts in edaphic conditions including soil temperature and moisture which dis-favored decay agents of crabgrass. On the other hand, rye residue may have served as an alternative food source to microbes which allowed for greater crabgrass seed survivorship. In addition, results showed that there was not a significant interaction between fungicide treatment and cover crop treatment suggesting that fungal pathogens did not mediate the effects of cover crops on seed persistence.

In summary, our results were not consistent with the hypothesis that weed seed persistence would be lower under conservation agricultural practices. In fact, persistence of seeds of both species was higher under ST compared with FWT and the persistence of large crabgrass was higher under rye cover cropping compared to no cover crop. Our results also did not support the hypothesis that tillage and cover crop effects on seed persistence were mediated by fungal pathogens. Rather, we found that tillage effects on persistence were explained

primarily by light exposure during tillage operations. This finding is potentially of great importance when interpreting studies evaluating the impact of tillage on seed persistence.

Our findings suggest that shifts in weed species density and composition under conservation agricultural practices are driven in part by differences in seed persistence. Although we found that light exposure was a major factor explaining tillage-induced changes in persistence, the mechanisms behind cover crop induced changes in persistence remain unclear. Future studies evaluating potential mechanisms responsible for the cover crop effects observed in this study would be particularly valuable for understanding and managing large crabgrass, a major weed problem in both conventional and conservation agricultural systems.

Tillage and Cover Crop Effects on Extracellular Enzymes of Soils and Seeds. We had hypothesized that winter rye cover crops and strip-tillage would result in greater microbial activity, and therefore greater extracellular enzymes, in the soil by providing a more suitable food source and stable habitat for microbes compared to conventional systems. Results from our research described in Chapter Four did show greater activity of BG, LAP, and PHOS under ST as well as the greater activity under a winter rye cover for BG and PHOS. Specifically we saw greater activity of enzymes associated with labile carbon, nitrogen, and organic phosphorous acquisition. These elevated activity levels under ST and a rye cover were temporally consistent which is different than other studies finding short-lived effects immediately following tillage (Lee et al. 1996; Lynch and Panting 1980).

We had also hypothesized that extracellular enzymes on seed surfaces would vary by weed species and be correlated with soil enzymes. We found that enzyme activity differed

between Powell amaranth and large crabgrass, especially on decayed seeds. Enzyme activities on large crabgrass seeds were often more than three times greater than activities on Powell amaranth seeds. This may have been due to inherent species differences or simply due to differences in seed surface area.

In general, we found few positive correlations between extracellular enzymes on seeds and those on the soil in which those seeds were buried. We had hypothesized that extracellular enzymes in both seeds and soils would be negatively correlated with the persistence of weed seeds in the soil. However, correlation analysis showed that the majority of significant relationships between soil enzyme activity and seed persistence were positive. Although caution must be exercised in inferring causation from such correlations, this result raises the question of whether elevated levels of certain soil enzymes reflect changes in soil microbial communities that are beneficial rather than detrimental to weed seed persistence. Finally, for Powell amaranth, decayed seeds had higher levels of certain enzymes than intact seeds and levels of those same enzymes on seeds were generally negatively correlated with seed persistence.

Implications. We found that ST and a winter rye cover crop decreased the short-term emergence of sown weed seeds but increased the long-term emergence of ambient weeds. In looking at short-term effects, our results suggest that fungal pathogens did not play an important role and that soil temperature, moisture, and nitrogen were likely more important in mediating management effects on weed emergence. Future studies evaluating the relative importance of these factors would be potentially useful.

Another important finding of our work was that ST and winter rye cover crops increased seed persistence as compared with FWT and no cover crop treatments and that the tillage effect was primarily explained by tillage-induced light mediation. This suggests that future studies addressing seed persistence and tillage dynamics will need to control for the effects of light. In addition, knowing that ST and cover cropping can increase seed persistence indicates that growers adopting conservation agricultural practices should be especially careful in preventing seed production within their fields.

Tillage and cover crop effects on persistence were not found to be mediated by fungal pathogens when using fungicide coated seeds but soil microbial activity, as measured by enzyme activity, was greater under ST and winter rye and may have played a role in explaining persistence in some cases. In general, microbial enzyme activity of either soils or seeds were not well correlated with seed persistence. However, in several instances, as soil microbial activity and seed microbial activity increased, seed persistence either increased or decreased, respectively. These results suggest it may be worthwhile for future studies to better investigate the relationships between the seed persistence of specific weed species and certain soil enzymes so as to gain insights into both weed seedbank community shifts and strategies to manage problematic species. In addition, microbial activities of soils and seeds were not well correlated and this spatial heterogeneity in microbial activity needs to be better understood by investigating how microbial activity surrounding buried seeds differs from the surrounding soil and what factors are mediating these differences.

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