

Physical and allelochemical cover crop effects for weed suppression

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Abstract

Winter annual weeds can delay soil warming, inhibit planting operations, and compete for water and nutrients resulting in yield loss of spring planted cash crop. Understanding the timing and extent of weed emergence in different cropping systems is important to producers to be able to predict occurrence and to better manage weeds. The first objective of this research was to model the emergence of winter annual weed species in two different cropping systems based on the accumulation of thermal time. Results show that winter annual weed species composition and emergence timing can vary significantly between locations and are highly site-specific. Certain weeds such as henbit had predictable and consistent emergence timings across years in a no-tillage system in eastern Kansas but was more variable in southeast Kansas. This information can be used by farmers for weed management decisions, such as timing of control methods. The use of cover crop monocultures and mixes were evaluated for their physical and chemical weed suppressive capabilities. The second objective was to assess the levels of physical weed suppression by each cover crop treatment through weed biomass and weed density at the time of cover crop harvest. Cover crop monocultures and mixes composed entirely or mostly of aggressive grass species were found to be the most weed suppressive due to their high biomass accumulation. Certain varieties of cereal rye, annual ryegrass, winter oat, and mixes containing oat and ryegrass were found to be the highest biomass producers. Overall, cover crops provided superior weed control relative to a fallow with herbicide treatment that had no residual activity. Fertility regimes can impact cover crop biomass production and influence their allelopathic potential. The third objective was to investigate the role of nitrogen and sulfur fertilizers on cover crop weed suppression through allelopathy by conducting a weed seed germination

bioassay. The results indicate that higher amounts of cover crop residues can potentially result in greater levels of weed suppression through inhibition of seed germination. Increasing soil fertility may decrease the allelopathic potential of cover crops, but can increase their biomass production, still resulting in adequate weed control.

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Chapter 1 - Literature Review

After the Green Revolution of the 1960's, food demand worldwide has been increasing exponentially (Lam et al. 2012). With a current world population of over 7.7 billion people that is expected to reach over 9 billion by the year 2050, the world's food production will need to increase by 70 to 100% of its current output to sustain such a population (Chauhan 2020). There are several biotic and abiotic constraints to crop production including but not limited to drought, floods, temperature extremes, soil degradation, environmental pollution, animal pests, pathogens, and weed infestations. Weed infestations are arguably the most important biotic constraints on crop production in both developing and developed countries worldwide along with pathogens and animal pests. Weeds ultimately compete for limiting resources such as sunlight, water, nutrients, and space that the crop would otherwise occupy, which results in yield loss.

Additionally, weeds can harbor detrimental crop pests such as insects, rodents, nematodes, mites, and pathogens such as fungi and bacteria. The amount of crop yield lost to weeds depends on the weed emergence time relative to the crop emergence time, the density of the weed population, and the species of the weeds and the crop. Weeds commonly reduce crop yield by 25% and if left uncontrolled can potentially result in 100% crop yield loss (Chauhan 2020, Lam et al. 2012). For example, weeds cost farmers in the US an estimated \$33 billion USD in lost crop production annually (Chauhan, 2020).

This has led to the increased use of herbicides to control weeds, which are now 37% of the total pesticides used worldwide. The overuse of herbicides results in pollutants in the soil, water, and aerial environments, and herbicide residue in food has deteriorated food quality (Lam et al. 2012). Furthermore, the continuous use of herbicides that utilize identical or similar modes of action (MOA) to control weeds has resulted in over 500 unique cases of herbicide-resistant

(HR) weed biotypes globally (Heap 2022). Of these 500 cases, over 160 are from the US, which is one of the highest number of cases in the world. Overall, there are now 260 species exhibiting herbicide resistance to 160 herbicides and 20 modes of action. The development of new herbicide MOAs are needed to manage HR weed biotypes, however, there has not been a new major MOA introduced within the last three decades, thus there is a need to develop and integrate different weed management options. Through improved herbicide technology, application timing, factor-adjusted dosages, precision agriculture techniques, and using herbicides with little known environmental impacts, farmers can reduce the risks of synthetic herbicides without lowering farm productivity (Tabaglio et al. 2013). Sustainable weed management also involves the integration of cultural techniques, such as proper crop rotation, tillage, narrow row-spacing, increased crop seeding rates, and the use of cover crops during the fallow period (Boyd et al. 2009, Buhler and Oplinger 1990, Tabaglio et al. 2013). Simply replacing synthetic herbicides with other direct weed control methods such as hand weeding and flaming, is labor intensive and usually results in inadequate weed control (Tabaglio et al. 2013). Therefore, utilizing an integrated approach using multiple, complementary tactics to manage weeds are widely considered as necessary to reduce the risk of herbicide resistance in weeds and to maintain crop productivity (Wallace et al. 2019).

Cover crops fit well into an integrated approach due to the various benefits they provide, such as erosion control, increased soil fertility, improved soil structure and water infiltration, reduced leaching and nutrient loss, enhanced biodiversity, and weed and pest management (Tabaglio et al. 2013). Cover crops are noneconomic crops that are generally classified by their life cycle, typically being either winter or summer annual, or perennial. The practice of cover cropping has been utilized for its various benefits for centuries, however, it was largely

abandoned in the 1940's and 1950's due to the advent of synthetic fertilizers and pesticides (Bergtold et al. 2017). With the resurgence of interest in sustainable agriculture in the 1980's and 1990's, along with the introduction of new cover crop species, tractor implements, and irrigation methods, farmers have been adopting cover crops into their crop rotations more frequently. Growing cover crops during the fallow season between cash crops has been increasingly adopted by farmers across conventional and organic cropping systems, but still only a small percent of US cropland is planted with cover crops (Dabney et al. 2001, Sturm et al., 2017). The fallow period, which lasts either 11 or 15 months, depending on the region and crop rotation, is an integral part of crop rotation in the semiarid regions of the US to store soil water for the subsequent cash crops (Holman et al. 2018). However, the fallow period comes with drawbacks such as increased operating costs, depletion of soil organic carbon, wind and water erosion, and soil degradation due to the lack of residue input. Cover crops also come with their own drawbacks, such as depletion of the soil water content in semiarid areas, such as the great plains region, leaving less soil moisture for subsequent crops (Holman et al. 2018). Thus, the choice of whether to plant a cover crop depends on the desired benefits, such as aiding in the suppression of HR weeds and for forage production. Ultimately, cover crops must increase subsequent crop yields through increasing nitrogen availability in the soil, improving the soil condition and health, and/or suppressing weeds to justify the expense of planting and growing them (Holman et al., 2018).

Cover crops are an important component of many integrated weed management (IWM) systems due to their competitive growth characteristics and biochemical effects on surrounding weeds. Many cover crop species are selected for their fast emergence, rapid canopy, root system development, and can generate a competitive crop environment for water, light, and nutrients

within four to eight weeks after sowing (Rueda-Ayala et al., 2015). Cover crops can reduce weed seed germination by producing dense canopies that shade the soil surface, which reduces the amount of available light and lowers the soil temperature (La Hovary 2011, Tabaglio et al. 2013). Furthermore, many cover crop species release allelopathic volatile compounds into the rhizosphere that can suppress weed germination and growth through root and shoot exudates, as well as residues left on the soil surface after termination (Cornelius & Bradley, 2017). If managed correctly, allelopathy has the potential to be used as part of an integrated weed management system (Bhowmik and Inderjit, 2003). In the midwestern US, winter hardy cover crops are planted in the fall, allowed to grow during the fallow period over the winter months and are then terminated the following spring prior to cash crop planting. Functional cover crops can be classified as cereals, legumes, brassicas, or other species, and they typically have different strategies to utilize the space and resources around them (MacLaren et al. 2019). Cover crops and weeds that have similar growth forms and root structures are more likely to be in direct competition for the surrounding limiting resources. The desired benefit of the grower depends on the cover crop planted; for example, leguminous cover crops can fix atmospheric nitrogen that increases the soil nitrogen availability during crop growth and through residue decomposition, however, the benefits of additional nitrogen added to the soil also comes with its drawbacks. A study conducted by Cornelius & Bradley (2017) found that cover crops of Austrian winter pea, hairy vetch, and crimson clover increased early season summer annual weed emergence by 36%, 31%, and 28%, respectively. Cover crop mixes have been gaining popularity as they can fulfill multiple functions while providing similar weed suppression to monocultures (Akemo et al. 2000, Baraibar et al. 2017, MacLaren et al. 2019).

Cereal and brassica species are particularly adept at capturing resources and rapidly accumulating aboveground biomass, which gives them the greatest competitive advantage over weeds. For example, Cornelius & Bradley (2017) found that cereal rye and winter wheat can reduce winter annual weed emergence by 53% and 50%, respectively. In addition, cereal rye can reduce early and late season summer annual weed emergence by 41% and 40%, respectively. Hayden et al. (2012) found that cereal rye reduced the biomass of winter annual weeds by 95 to 98%, a significant reduction. Another study conducted by Al-Khatib et al. (1997) found that rapeseed residues incorporated into the soil in the spring after termination can reduce weed densities and biomass by 73 to 85% and 50 to 96%. Fall-planted cereal cover crop species tend to have higher levels of winter annual weed suppression due to their fast emergence and growth, their winter hardiness, and their higher levels of ground cover relative to other species of cover crops. Not only do cover crops reduce weed biomass in many agricultural production systems, but they can also reduce the density of weed seedbanks in the soil and the survival success of HR weeds (Nichols et al. 2020). The interference of annual weeds on crops, which are most of the problematic weeds in the midwestern US, is often due to the replenishing weed seedbank which can persist for several years. This creates a ‘legacy effect’ that renders any short-term weed management tactics negligible. A study conducted by Nichols et al. (2020) revealed that modest amounts of cover crop biomass over the winter can provide enough ground cover to increase weed seed mortality through granivore activity. Furthermore, the allelopathic compounds from rye residue can potentially catalyze pathogen attacks on weed seeds, thus reducing the vigor of germinated seedlings (Barnes and Putnam 1983, Mohler et al. 2012).

In the US, summer annual weeds are responsible for most of the corn and soybean yield losses because their life cycles overlap, resulting in direct competition (Cornelius & Bradley,

2017). Weed communities result in yield loss often due to their time of emergence, as negative competitive effects are more apparent if plants germinate and grow during the same time (Nichols et al., 2020). However, winter annual weeds are becoming increasingly common in many cropping systems throughout the midwestern US and are often overlooked because they complete their lifecycle by the time of crop sowing (Werle 2012). Tillage systems can have a major influence on weed populations, as not all systems have winter annual weed problems. Conventional tillage systems will typically have reduced annual weed densities, whereas herbicide selection becomes more important in reduced or zero tillage systems (Buhler and Oplinger, 1990). The dense vegetative mats produced by winter annual weeds can have several detrimental effects on the success of subsequent cash crops by delaying soil warming, competing for nutrients and water and impeding planting operations (Werle 2012). Winter annual weeds such as henbit (*Lamium amplexicaule* L.) are also known to be alternate hosts for several pests, such as the soybean cyst nematode (*Heterodera glycines*, SCN) and the two-spotted spider mite (Woolam et al. 2018). Understanding the timing of winter annual weed emergence is critical to ensure that control measures are implemented at the early stages of growth. Herbicide applications in late fall or early spring have become more common to control winter annual weeds. Fall applications are effective because the weeds are relatively small and therefore more susceptible to herbicides, however, environmental conditions may not be ideal for herbicide uptake (Monnig and Bradley, 2007). On the other hand, spring herbicide applications are less efficient because the weeds are at a more advanced growth stage and are larger in size, which results in inadequate control (Woolam et al. 2018). Hence, a better understanding of the germination and emergence patterns of winter annual weeds can be useful in creating models that can aid in farmers developing weed management programs for their fields.

Winter annual weeds generally emerge in the fall, overwinter as small seedlings, followed by fast growth in the spring and seed production and senescence in late spring or early summer. They can be broken into two distinct groups, strict versus facultative winter annuals; strict winter annuals mostly germinate during late summer into early fall, when the soil temperatures are decreasing, as the high summer temperatures have broken their dormancy (Baskin and Baskin, 1988, Werle et al. 2014). Facultative winter annual weeds can germinate in the fall or early spring, depending on the environmental conditions. This may be advantageous to the survival and reproduction of the plants due to a decreased risk of severe winters killing the entire population. Facultative winter annuals can delay germination until environmental conditions are more suitable to increase the chance of offspring reaching reproductive maturity (Werle et al. 2014). In terms of control, strict annuals are best managed in the fall after most of the seedlings have emerged, whereas facultative annuals should be controlled in early spring. The composition of winter annual weeds in any given location is ultimately a function of the species present in the soil seedbank and their interaction with the environment. Seed rain events result in a soil seedbank that can persist for several years, thus, knowing what weed species are present in the soil seedbank and when they are likely to emerge is critical in the development of weed management programs (Grundy 2003).

Allelopathy can be defined as the process involving secondary metabolites that are produced by plants, microorganisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (Cheng and Cheng, 2015). These secondary metabolites are known as allelochemicals and can have either positive or negative effects on target organisms, however, allelopathy is often referred to as a negative interaction (Shirgapure and Ghosh, 2020). Whether the effect is positive or negative is subjective and depends on the

context of the interaction. For example, positive effects of allelochemicals for the plant producing them include the attraction of beneficial pollinators or seed dispersers, and defense mechanisms over natural predators that aid in the plant's survival. Potential negative effects of allelopathy could be the inhibition of crop seed germination, reduction of vegetative growth and crop yield (Cheng and Cheng, 2015, Shirgapure and Ghosh, 2020). The allelochemicals can be broadly classified into one of fourteen categories based on chemical similarity such as straight-chain alcohols, water-soluble organic acids, simple unsaturated lactones, benzoquinones, cinnamic acid and benzoic acid to name a few. Within the context of this paper and cover crops, two of the most prominent allelochemicals are benzoxazinoids found in cereals, and glucosinolates found in brassicas (Norsworthy et al 2005, Schulz et al. 2013).

The most potent allelochemicals found in members of the *Poaceae* family are glycosylated benzoxazinonones (BX) (La Hovary 2011). The two critical BX's found in rye and wheat are DIBOA [2, -dihydroxy-1,4 benzoxazin-3-one] glucoside predominantly found in the shoots and DIMBOA [2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one] glucoside found in the roots (Lam et. al. 2012, Schulz et al. 2013). These two compounds often occur together with their biochemical degradation products, known as benzoxazolinones, BOA [2-(3H)-benzoxazolinone] glucoside and MBOA [6-Methoxy-2-benzoxazolinone] glucoside. These glycosylated end products are stored in the vacuoles of cells within the young tissues of the plant's roots and shoots and it has been found that the BX biosynthesis is at its highest in the tissues of young rye plants, which decreases over time during plant development (Schulz et al. 2013, La Hovary 2011, Macias et al. 2005). Benzoxazinones are nontoxic while in their glucoside form in the vacuole of plant cells, but are hydrolyzed by the enzyme β -glucosidase upon destruction of the cell membrane through plant wounding or tissue death. This enzyme

transforms the BX's from their non-phytotoxic glucoside form into aglucones, which are toxic compounds to other organisms (Wouters et al. 2016). Brassicaceae plants, on the other hand, have high levels of glucosinolates within their tissues. The production and storage of these compounds like BX's, and they are hydrolyzed into isothiocyanates, which suppress various weeds and soil pests (Norsworthy et al. 2005).

Plants have several methods of releasing allelochemicals into the surrounding environment, including volatilization, root exudation, and leachates from living or decaying plant tissue. One of the main mechanisms of allelopathy is through root exudation, where the plant releases allelochemicals into the soil which are then absorbed by the target plant and translocated into the xylem. Inhibition of seed germination, seedling growth, chlorophyll content and respiratory activity are severely affected in neighboring plants by allelochemicals (Chiapusio et al. 2003, Wouters et al. 2016). The result of allelopathy on the target plant is often inhibition of germination and reduced seedling growth due to membrane damage caused by lipid peroxidation, which affects processes integral to plant growth such as photosynthesis, electron transport, and protein synthesis (Chiapusio et al. 2003, Schulz et al. 2013, Wouters et al. 2016). The degree of phytotoxicity vary depending on the relative concentration of the allelochemical, the rate of translocation into the target plant, the microbial degradation processes in the soil, and the resistance of the target plant to allelochemicals through reduced uptake and detoxification mechanisms (Macias et al. 2005, Wouters et al. 2016). In addition, the target plant's seed size is a prominent factor in its susceptibility to allelochemicals, with small-seeded plants often being more prone to allelopathy than large-seeded plants (Burgos and Talbert 2000, Flood and Entz 2009, Leibman and Sundberg 2006). Seed germination bioassay experiments conducted by Burgos and Talbert (2000) found that rye residue leachates could have potential in controlling

small-seeded weeds, such as Palmer amaranth (*Amaranthus palmeri* S. Watson), large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and prickly sida (*Sida spinosa* L.), within large-seeded crops such as corn (*Zea mays* L.) and many *Cucurbitaceae* species.

Allelopathic compounds are produced in differing quantities in plants depending on a variety of biotic and abiotic factors. The production of allelochemicals can vary within the same species of plant through hybrids or cultivars and with plant maturity. For example, Burgos et al. (1999) tested the allelopathic potential of eight different rye cultivars at two termination times in field and greenhouse settings. The findings from this study were that ‘Bonel’ rye and ‘Aroostok’ rye were two of the highest producers of allelochemicals on a per-gram of shoot tissue basis. Another finding from this study was that the highest concentration of allelochemicals in the plant tissues was between 30 and 60 DAP, or in the young plant tissues. The caveat with this information is that even though ‘Bonel’ and ‘Aroostok’ had high concentrations of allelochemicals, they were some of the lowest biomass producers among the other rye cultivars, somewhat hindering their weed suppressive capabilities (Burgos et al. 1999). In addition, biotic stress factors such as plant diseases, insect damage, herbivory, and abiotic stress factors such as high temperature, and low nutrient and moisture availability can all enhance the biosynthesis of allelochemicals (Schulz et al. 2013). This is hypothesized to be an adaptive mechanism in response to poor growing conditions that will increase the competitive ability of the plant and thus its survivability. Mwaja et al. (1995) conducted a modified Parker bioassay experiment using the dried biomass of ‘Wheeler’ rye and hairy vetch (*Vicia villosa* Roth) that had been grown under low, medium, and high fertilizer rates. The results from that study showed that the rye and vetch produced more biomass in response to the high fertilizer rate, but the extracts from

the dried shoots were less inhibitory to cress (*Lepidium sativum* L.) germination and radicle elongation at that rate.

The use of fertilizers may not increase the allelochemical content within rye, but the increased biomass production in response to higher fertility could be a useful method for weed suppression if the residues are left on the soil surface. For example, Tabaglio et al. (2013) found that the effect of 250 and 300 kg ha⁻¹ nitrogen applications increased rye biomass from 81% to 135% respectively, compared to the zero-nitrogen control. The allelochemicals released into the soil from the rye residues left on the soil surface in no-till plots were 57% and 105% higher than the unfertilized plots. This resulted in a 61% reduction in grass species germination, and a 96% reduction of broadleaf species germination (Tabaglio et al. 2013). Rye residues left on the soil surface in reduced or no-till systems can release allelochemical leachates into the soil for a significantly longer period than the residual effects of the allelochemicals themselves (Macias et al. 2005). Macias et al. (2005) found that DIBOA transforms into BOA between 2 to 10 days and is degraded by microorganisms within 45 days. Rye residue, however, can remain on the soil surface for up to 60 days, releasing allelochemicals throughout the degradation process. This provides a slow-release form of bioherbicide which in combination with the physical effects of plant residue decreasing weed germination, can suppress weeds in organic based cropping systems (Macias et al. 2005, Tabaglio et al. 2013). Overall, rye cover crops included in rotation may be a sustainable weed management strategy that could reduce herbicides used in agroecosystems.

Sulfur is another essential element for plant growth and is an important limiting factor for crop production since the enactment of the Clean Air Act of 1970, which reduced the amount of sulfur dioxide in the air (Carciochi et al. 2017). This factor, in combination with low soil organic

matter, soil erosion, and high nutrient loss rates through crop residue removal has resulted in sulfur shortages. Sulfur is important for increasing the nitrogen recovery efficiency, that is, the amount of nitrogen absorbed by the crop per unit of nitrogen applied, and the nitrogen internal efficiency, which is the biomass or crop yield produced per unit of nitrogen (Carciochi et al. 2017, Salvagiotti et al. 2009). Carciochi et al. (2017) found that the shoot mass and nitrogen use efficiency of wheat plants were greater under the addition of sulfur fertilizer. Little research has been conducted on the effect of sulfur fertilizer on cover crop allelopathic potential, given that it may increase the biomass production of plants by increasing the nitrogen use efficiency, it may enhance the weed suppressive capabilities of cover crops. Research has been conducted on the detoxification of BX's by plants grown under varying sulfur conditions. Knop et al. (2006) found that sulfur deficiency alone did not affect the detoxification of BOA in plants, however, a sulfur deficiency with an application of S-metolachlor reduced the detoxification of plants by 60%.

Weed management is the most difficult challenge for organic farmers and replacing synthetic herbicides with other direct control methods usually results in inadequate weed control. Thus, weed management should be viewed as a component of integrated crop management in which crop rotation, seeding rates and row spacing, cover crops and allelopathy, and tillage in conventional cropping systems are utilized (Boyd et al. 2009, Schulz et al. 2013). Cover crops fit well into an integrated approach to weed management due to the various benefits they provide. In addition, fertilization regimes can also be altered to attain greater amounts of cover crop biomass, thus increasing the physical and chemical suppression of weeds. The research provided in this study can be helpful in better understanding the weed suppressive effects of various cover crops in two different cropping and tillage systems. Recording the emergence patterns of the weeds within these different systems will provide more data to be used in developing emergence

models, which can then be used by farmer to better be able to predict and control troublesome weeds in their fields.

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Chapter 2 - Effects of Cover Cropping on Weed Management in a Corn to Soybean Crop Rotation in Southeast Kansas

Abstract

Winter annual weeds can establish in the fall and interfere with field operations and reduce yield of spring planted cash crops through increased competition for limiting resources. Cover crops have the potential to improve long-term weed population management and reduce weed pressure on cash crops. In addition, gaining a better understanding of the timing and extent of winter annual weed emergence is critical in helping farmers manage weed infestations. Field experiments were conducted on a conventional tillage corn to soybean crop rotation in Parsons, Kansas, from 2020 to 2022 to evaluate the effects of ten different cover crop treatments on weed population density and biomass. Weed species composition, relative abundance, and emergence patterns were documented in P.V.C. weed rings placed within the cover crop treatments throughout the experiment. Weed biomass and densities were collected at the time of cover crop termination in late spring to early summer. All cover crop treatments reduced weed biomass by 20% to 99% compared to the fallow with fall herbicide control treatment. Winter wheat, drilled radish and ryegrass, “Deer mix”, and winter oats were among the most weed suppressive cover crops, reducing weed biomass by 98% to 99% compared to the fallow control. Forage collards was one of the least weed suppressive cover crop treatments, reducing weed biomass by 42%, and the fallow with herbicide was the least weed suppressive. Dominant weed species observed in both years were little barley (*Hordeum pusillum* L.), henbit (*Lamium amplexicaule* L.), and common chickweed (*Stellaria media* L. Vill.). Planting aggressive grass species such as oats and

wheat in cover crop mixtures can dramatically reduce the establishment and growth of weeds throughout the fallow period, thus reducing competition for early spring or summer cash crops.

Introduction

Weed suppression by cover crops has become more important as a part of an integrated weed management (IWM) program to improve long-term weed population management and reduce weed pressure on cash crops. Integrated weed management programs often utilize a combination of synthetic herbicides (Wallace et al. 2019, Monnig and Bradley 2007), cover crop sowing date and seeding rates (Sturm et al. 2017, Boyd et al. 2009), tillage practices (Buhler and Oplinger 1990), and various fertilization regimes (Little et al. 2021, Kumar and Jha 2016) to control weed infestations in crops. Winter annual weeds can establish over the fallow period and then directly compete with early season or summer cash crops, resulting in delayed establishment and potential yield losses (Werle et al. 2014). In addition, winter annual weeds that are not controlled can increase the size of the soil seedbank, leading to persistent weed infestations over time (Travlos et al. 2021). Weed suppressive cover crops have been shown to reduce weed biomass and weed seed production in many cropping systems, including corn (*Zea mays* L.) to soybean (*Glycine max* (L.) Merr.) rotations, reducing the overall weed population growth and competitive pressure on cash crops (Baraibar et al. 2017, Nichols et al. 2020). Different cover crop species can provide multiple ecosystem functions, such as nitrogen fixation with legumes, and can be planted as either monocultures with one species, or as a mixture containing multiple species (McLaren et al. 2019). The weed suppressive capabilities of different cover crop species can vary, thus, the objective of this study was to determine what cover crops consistently provided the best weed suppression in terms of lower weed biomass and weed density over two growing seasons in a typical corn to soybean crop rotation in southeast Kansas.

Cover crops are noneconomic crops that are typically planted in rotation with other field crops for the substantial benefits they provide. Because they are often planted during the fallow

period between cash crops, they are commonly defined as crops grown to cover the ground and protect from soil erosion and loss of nutrients through leaching and runoff (Dabney et al. 2001). Weed suppressive cover crops exhibiting fast emergence and growth, winter hardiness, and greater levels of ground cover are an important component of integrated weed management to prevent weed population increases and prevent cash crop yield loss (Hayden et al. 2012). This is especially important for corn to soybean crop rotations in the US, where one third of the world's total corn and soybean production originates from (Nichols et al 2020). Current estimates of economic losses due to weed is \$33 billion USD annually and farmers spend as much as \$6 billion USD annually on weed control measures, such as synthetic herbicides and equipment for tillage and field cultivation (Chauhan 2020). In addition, the overreliance on herbicides for weed control has increased the occurrence of herbicide-resistant weeds, with over 500 unique cases of herbicide resistance globally (Heap 2022). Integrated weed management utilizing multiple, complementary tactics to manage weed populations is considered necessary to reduce the risk of increasing herbicide resistance in weeds. Cover crops have been proposed to reduce weed emergence and growth over the fallow period, along with the various benefits they provide other than weed control. Aside from reducing erosion, cover crops can also increase the nutrient use efficiency of crops, reduce pesticide use through increased weed suppression, increase the soil organic matter and soil porosity, and even increase inoculum of beneficial mycorrhizal fungi in the soil (Dabney et al. 2001).

Winter annual weeds can result in yield losses, particularly in reduced or no-till systems. In these conservation tillage systems, fall-emerging weeds can establish a strong root system, accumulate rapid biomass in the spring and compete directly with early spring or summer cash crops (Werle et al. 2014). They can also interfere with planting operations, delay soil warming,

and harbor detrimental pests such as the soybean cyst nematode. Additionally, winter annual weeds that are not controlled can increase the soil seedbank and can act as ‘biological bridges’ for parasitic nematodes and plant pathogenic viruses from one season to the next (Nichols et al 2020). Living cover crops or cover crop residues provide habitat for beneficial insects that feed on the weed seedbank, thus reducing seedbank size. Winter annual weeds can provide some ecosystem functions, such as erosion control and increased nutrient cycling, and are often controlled in conventional cropping systems through pre-plant tilling and herbicide applications (Hayden et al. 2012). In addition, areas where rainfall is sparse and water concerns are high, keeping the fields fallow is a common practice to store soil water for subsequent crops (Holman et al. 2018). Most producers plant cover crops primarily for HR weed control and for forage production. Therefore, the decision to plant cover crops is largely an economic one, in which the farmer must balance the advantages and disadvantages, including the biophysical benefits of including cover crops in rotation.

The level of weed suppression provided by cover crops can be attributed to their fast emergence in the fall and their rapid above and below ground biomass accumulation, which creates a competitive environment for weeds to grow in (Reuda-Ayala et al. 2015). Cover crops can displace weeds through resource competition and niche disruption while they are actively growing, and through the phytotoxic effects of their residues, which can provide more weed control long after they are terminated for cash crop planting (Al-Khatib et al. 1997, Creamer et al. 2021). Cover crops are useful components of an IWM approach that can help to reduce the farmers reliance on herbicides, thus decreasing the risk of selecting for HR weed populations (Heap 2022). Utilizing cover crops to reduce the density of weed populations and the number of large individual weeds at the time of post herbicide applications are just a few of the weed

management goals of including cover crops in rotations. For example, Wallace et al. (2019) discovered that the inclusion of cereal rye or rye plus forage radish reduced horseweed (*Erigeron canadensis* L.) density by as much as 86% by the time herbicides were applied. Reducing the size of individual weeds and the density of the population, better weed control can be attained through herbicide application. Large weeds, particularly at the time of herbicide exposure can result in inadequate control and increase the risk of weed populations developing HR (Heap 2022). Cover crops can also have significant effects on the weed seedbank size, which is one of the primary mechanisms by which problematic weeds in the Midwest persist for years. A study conducted by Nichols et al. (2020) found that the weed seedbanks of greater than 300 seeds m⁻² consistently had lower weed seedbank densities after cover crop growth, compared to the no-cover control.

Many studies have been conducted on cover crop species mixtures, with an emphasis on utilizing multiple plant species with complementary growth characteristics. The concept behind this is that by increasing the plant diversity, you can enhance certain ecosystem functions and increase crop productivity, increase soil carbon storage, and increase nutrient cycling (MacLaren et al. 2019). Plants vary in their resource requirements and their functional abilities to acquire such resources; thus, more diverse plant mixes can have complementary strategies for resource capture. Cover crop mixtures in theory may potentially be more weed suppressive due to the diversity-invasibility hypothesis, which states that the more diverse a biological system is, the less likely it is to have an invasion of exotic species (Smith et al. 2020). Studies have shown that the effect of cover crop mixes on weed suppression is largely due to the inclusion of one dominant species that accumulates a large amount of aboveground biomass. For example, Baraibar et al. (2017) found that cereal rye accounted for at least 80% of the spring harvested

biomass in cover crop mixtures including Austrian winter pea (*Pisum sativum* L.), red clover (*Trifolium pratense* L.), and canola (*Brassica napus* L.). Similarly, Hayden et al. (2012) found that “Wheeler” cereal rye alone and rye mixed with hairy vetch (*Vicia villosa* Roth.), were more weed suppressive than vetch alone. A study conducted by MacLaren et al. (2019) looking at different cover crop mixture proportions of “Wheeler” rye and field pea found lower weed biomass in the cover crop mixes composed mostly of rye versus pea. In conclusion, planting cover crop mixtures that contain highly productive species, such as certain varieties of cereal rye, may yield better weed suppression than monocultures of legumes or brassicas. The benefits of including other functional cover crop species in mixtures is through other ecosystem functions, such as nitrogen fixation.

The weed-suppressive potential of six cover crop monocultures and three cover crop mixtures compared to a no-cover control with fall herbicides were assessed in this study in southeast KS through the collection of cover crop and weed biomass and weed density at cover crop termination. Weed emergence was documented within the cover crop treatments to gain a better understanding of the timing and extent of emergence, which can be helpful to farmers on weed management decisions.

Materials and Methods

2.1: Field Descriptions and Experimental Design

Field experiments were conducted fall 2020 to spring 2021 and fall 2021 to spring 2022 in Labette County at the Kansas State University Southeast Research and Extension Center (SEREC) near Parsons, Kansas (37°21'54.48"N, 95°17'15.27"W, 178 m elevation). The monthly average rainfall and temperatures are presented in Table 2.1. The soil type was a Parsons silt loam (Fine, mixed, active, thermic Mollic Albaqualfs) with 2.1% organic matter and a pH of 5.6 in 2022 and is an upland soil with a clay pan. The experiment was conducted in a randomized complete block design with four replications of the 10 cover crop treatments including six cover crop monocultures, three cover crop mixtures and a no-cover chemical fallow treatment. The individual plots measured 3 m by 12 m and the entire experimental area was surrounded by 9 m alleys planted to wheat. Cover crop species composition and seeding rates are presented in Table 2.2. The cover crops were planted into fields that had previously been planted with corn, followed by soybean being planted after the cover crops with conventional tillage and no supplemental irrigation. Cover crops were either planted with a drill seeder (John Deere 750, Deere & Company, 1 John Deere Place. Moline, IL 61265) at a depth of 2.54 cm with rows spaced 19 cm apart or broadcast seeded with an air seeder attachment and then incorporated into the soil with light tillage. The no-cover chemical fallow treatment received a fall application of glyphosate at 0.98 kg ha⁻¹ plus dicamba at 0.42 kg ha⁻¹. Cover crops were terminated from late spring to early summer with herbicides and then the field was tilled to incorporate the cover crop stubble into the soil (Table 2.3).

Within two weeks of the cover crops being planted in the fall, weed emergence was recorded from two permanent P.V.C. rings (d = 20.3 cm) placed within each cover crop plot.

Within one of the rings, weed species were identified, enumerated, and removed by hand on a bi-weekly basis until cover crop biomass collection the following spring (Table 2.3). Emerged seedlings were identified and pulled when the cotyledons were fully expanded to ensure accurate identification of the seedlings and minimal soil disturbance. The other ring was used for comparison as the weeds were identified and enumerated, but not removed. Weed and cover crop biomass were sampled before cover crop termination in late spring or early summer by randomly placing one 0.25-m² quadrat in each plot over three crop rows of the drill-seeded cover crops. Weed density was determined by identifying and enumerating the individual plants within the quadrat per treatment per replication. All cover crop and weed biomass was clipped at ground level within the quadrat and placed in separate paper bags. The paper bags were then placed in a drying oven at 60°C for 96 hours and weighed to determine final dried biomass. Weed biomass sample from each plot was combined across species in 2021, whereas weed biomass samples were documented separately for each species and added together for each plot in 2022. Dates of major field operations, P.V.C. ring installation, and cover crop biomass collection are in Table 2.3.

2.2. Weed Flora and Emergence

The daily maximum and minimum air temperatures, precipitation, and 5 cm depth soil temperature were obtained from the nearest weather station in Parsons, KS (Kansas Mesonet; site ID: MTNK1; 39.209° N, 96.59° W). The weed emergence data were converted from bi-weekly counts to cumulative counts, and then to cumulative emergence (%) based on the final total plant emergence per ring per year. Cumulative emergences were pooled over cover crop treatment and replication for each weed species per year and emergence curves were constructed for each weed species per year using the 5 cm soil thermal time (TT) units. For winter annual weeds,

cumulative soil thermal time was calculated for each day after August 1 of each year the experiment was conducted. August 1 was chosen as the start date of accumulation of soil TT units based on previous studies and the fact that winter annual weeds are released from dormancy by high summer temperatures and germinate when temperatures start to gradually decrease (Baskin and Baskin, 1988). Cumulative soil TT was calculated as:

$$TT = \sum_{i=1}^n (T_{mean} - T_{base})$$

where T_{mean} = daily mean soil temperature at 5 cm depth (C), T_{base} = base soil temperature for each species for seedling emergence (C).

The base temperatures for germination used in the equation were 0 C for purslane speedwell (*Veronica peregrina* L.), mousetail (*Myosurus minimus* L.), small-leaved bittercress (*Cardamine parviflora* L.), field pennycress (*Thlaspi arvense* L.), and blue mustard (*Chorispora tenella* (Pall) DC.), 1.4 C for common chickweed (*Stellaria media* L. Vill.), and 2 C for henbit (*Lamium amplexicaule* L.). Little barley (*Hordeum pusillum* L.), the only short-lived perennial weed observed in this experiment, cumulative TT units were calculated starting January 1 of each year, and the germination threshold value used in the equation was 6 C (Werle 2012). Weed counts were modelled using a logistic function to describe cumulative emergence by species;

$$y = \frac{L}{1 + e^{-k(x-x_0)}}$$

where: y is the cumulative emergence (%) as a function of cumulative soil TT, x_0 equals the cumulative soil TT value of the logistic function midpoint, L is the maximum cumulative emergence, and k is the logistic growth rate or steepness of the curve (Saini 2021).

Each individual weed species was grouped into strict winter annuals versus facultative winter annuals by summing the number of emerged seedlings from the weed pull rings before and after January 1. These weed count sums were then converted into a percent of total before and after January 1. If the majority (>70%) of the seedlings emerged before January 1, then they were classified as mostly fall-emerging or strict winter annuals. If the proportion of seedlings emerged before and after January 1 was nearly even (50:50), then they were classified as fall and spring emerging winter annual weeds. If the majority (>70%) of seedlings emerged after January 1, they were classified as facultative winter annual weeds (Baskin and Baskin 1988, Cici and Van Acker 2011).

Analysis of weed flora and diversity were determined by calculating the proportion of each weed species as a percentage of the total counts for both the weed pull ring counts and the quadrat counts taken at cover crop termination. The numeric abundance and distribution of individual weed species in the weed flora of the plots were calculated using four quantitative measures adapted from Thomas (1985): Frequency (F), Relative Frequency (RF), Relative Density (RD), and Relative Abundance (RA).

Frequency value is the presence (1) or absence (0) of species k in each weed pull ring.

Relative Frequency for species k in each weed pull-ring or 0.25-m² quadrat (RF_k) =

$$\frac{\text{Frequency value of species } k}{\text{Sum of frequency values for all species}} * 100$$

Relative Density for species k in each weed pull-ring or 0.25-m² quadrat (RD_k) =

$$\frac{\text{Total counts for species } k}{\text{Sum of counts for all species}} * 100$$

Relative Abundance for species k in each weed pull-ring or 0.25-m² quadrat (RA_k) =

$$\frac{RD_k + RF_k}{2}$$

2.3. Data Analysis

All data were analyzed using the PROC GLIMMIX procedure in SAS (v. 9.4, SAS Institute, Cary NC). The models were checked for normal distribution and equal variances and pairwise comparisons within fixed effects observed with the LSMEANS statement. Weed count and relative abundance data were analyzed by weed species and cover crop treatment including all interactions. Relative abundance values were calculated for both the ring weed counts and the 0.25-m² quadrat weed counts separately. If a weed species appeared in one year and not the other, it was omitted from the ANOVA analysis between years in order to get a clear comparison. Biomass data were analyzed separately by either cover crop or weed biomass by cover crop treatment. Weed biomass was pooled by species over cover crop treatment in 2021, thus only cover crop biomass and weed counts were analyzed across years. If the two- or three-way interactions were not significant at P<0.05, further analyses were performed for two-way interactions and/or fixed effects. Cover crop was considered a fixed effect, while year and replication were considered random effects. The weed counts were modelled using a logistic function to describe cumulative emergence by species over cumulative soil TT. Logistic model, function parameters, and emergence graphs generated by GraphRobot (Wang 2019).

Results and Discussion

2.1. Weed Species Composition and Emergence

Throughout both experimental years, a total of eight weed species with varying levels of relative abundance were observed in the weed pull rings (Table 2.4). Six of them occurred in both years, including henbit, little barley, small-leaved bittercress, common chickweed, blue mustard, and field pennycress. Two other weed species were observed with mousetail in 2021 and Purslane speedwell in 2022. An analysis of the relative abundance of the six consistent weed species across years showed that the interaction between weed species and cover crop treatment was significant. Years will be explored separately.

The 2021 two-way ANOVA with weed species and cover crop treatment as the two factors revealed that the interaction was not significant. There were no differences in relative abundance due to cover crop treatment, but there were differences among the weed species. The top four species were little barley, henbit, common chickweed, and small-leaved bittercress in terms of percent composition ranging from 28.5% to 17.8% and relative abundance ranged from 23.9 to 20.1 (Table 2.4). In 2022, the two-way ANOVA interaction of cover crop treatment by weed species was significant. The top four species were little barley, henbit, common chickweed, and purslane speedwell ranging from 46.9% to 10% of the total composition, and relative abundance ranging from 25.8 to 13.5 (Table 2.4). Field pennycress and blue mustard were two of the least abundant weeds seen in both years, accounting for less than 10% of the total composition and relative abundance in each year (Table 2.4). Small-leaved bittercress was seen at a greater percent composition and abundance in 2021 than in 2022.

Within the 0.25-m² quadrats from 2021 and 2022, there were a total of nine weed species observed at the time of cover crop termination (Table 2.4). Four of the weed species were

consistent across years, including common chickweed, little barley, blue mustard, and small-leaved bittercress, whereas field pennycress was only observed in 2021, and purslane speedwell, henbit, mousetail, and horseweed were observed in 2022. An analysis of the relative abundance of the four consistent weed species across years showed that the interaction between weed species and cover crop treatment was not significant. Years will be explored separately.

Within each year, a two-way ANOVA of cover crop treatment by weed species at time of termination revealed that weed species was significant. The 2021 weed species composition was dominated by common chickweed and little barley accounting for 36.4% and 31.5% of the total, respectively, and relative abundance values of 33.2 and 27.3, respectively. Small-leaved bittercress, field pennycress, and blue mustard were observed less frequently, ranging in percent composition from 16.8% to 7.2% and relative abundance ranging from 17.1 to 9.8 (Table 2.4). In 2022, purslane speedwell, little barley, and henbit were the dominant weed species, accounting for 38.9%, 37.5% and 19.3% of the total composition, respectively, and relative abundance values of 37.2, 27.9, and 25.2, respectively. The least observed weed species were common chickweed, small-leaved bittercress, horseweed, mousetail, and blue mustard, with percent composition ranging from 3.1% to less than 1%, and relative abundance values ranging from 12.6 to 0.3. The composition and abundances of the weeds observed most likely reflects the composition of the seedbank, which is often replenished by seed rain events by annual weed species (Nichols et al. 2020). Some of the low relative abundance and composition (%) weeds were much larger than the high relative abundance and composition (%) species. For example, most of the little barley plants counted within the quadrats were very small, less than three cm in height, whereas the horseweed plants were much larger, measuring near 12 cm in height in some cases. There was not a lot of ground cover achieved by the high relative abundance weeds, so

competitive exclusion of the less abundant weeds is not very likely. The species diversity captured within the quadrat was likely due to random placement within the plots and a larger sampling area.

Weed species emergence profiles were developed for henbit and for common chickweed based on observations in the 2021 to 2022 season (Figure 2.1). The rate of exponential growth of the logistic curve was higher with common chickweed ($k=0.005$) versus henbit ($k=0.004$), indicating that the emergence timing of common chickweed was faster than henbit. Half of the henbit and common chickweed seedlings emerged in the fall and half in the spring, indicating that they were facultative in nature and were not behaving as strict winter annuals (Figure 2.2). Purslane speedwell seedling emergence was 37% in the fall and 63% in the spring and was categorized as a facultative winter annual, albeit a mostly spring germinator. Field pennycress, small-flowered bittercress, and blue mustard seedlings emerged predominantly in the fall with 90%, 80%, and 77% emerging before January 1, respectively, placing them in the strict winter annual category (Figure 2.2). Little barley had 83% of the seedlings germinate in the spring, so it was also placed in the facultative winter annual group. Climate and weather define the emergence patterns for each weed species, which are region specific. Although winter annual weeds typically have consistent emergence patterns across years, some of the weeds observed in this study can act as more strict winter annual species that germinate mostly in the fall, or facultative winter annual species that germinate mostly in the spring, or half in the fall and half in the spring.

Studies on winter annual weed emergence have found that henbit behaves as a strict winter annual in Louisiana (Woolam et al. 2019), and in central Nebraska (Werle et al. 2014, Werle 2012). Henbit emergence in Parsons, KS, was facultative in nature, with half emerging in

the fall and half in the spring. Field pennycress germination also displayed a facultative emergence pattern similar to henbit, whereas it has been observed as a mostly spring germinator in Nebraska (Werle et al. 2014). Baskin and Baskin (1988) reported that when the seeds of certain winter annuals such as henbit, common chickweed, and field pennycress remain ungerminated by the end of the autumn germinating period, they may enter conditional dormancy after being exposed to low winter temperatures. This results in seeds losing the ability to germinate at high temperatures but gaining the ability to germinate at low temperatures. The result of this is that they act somewhat like spring ephemerals, germinating in early spring. The differences in germination between years in this study can be largely explained by the local weather patterns, as temperature is one of the primary environmental factors regulating germination (Baskin and Baskin 1988). Weed seedbanks are often the primary source of persistent weed infestations (Travlos et al. 2020), and Parsons experienced below average temperatures particularly in January and February, potentially inducing conditional dormancy. Temperature fluctuations have also been reported to increase germination and emergence rates of certain winter annual weeds (Werle et al. 2014). In general, seasonal environmental patterns and how it relates to weed emergence are often highly location dependent, thus emergence data are useful and applicable in the region where the experiments were conducted.

2.2. Cover Crop Treatments and Weed Suppression

Interaction of year and cover crop treatment was not significant for total number of weeds counted within the pull weed rings. The main effect of year was a significant factor but cover

crop treatment was not, therefore, data will be presented across cover crop treatments by year (Figure 2.3). A further analysis of mean weed counts by cover crop within year showed that for 2021 and 2022, weed counts did not differ between the cover crop treatments.

A comparison of the cover crop biomass across both experimental years yielded significant results for the interaction, mainly more biomass in 2021 as compared to 2022 because cover crop biomass harvest occurred in early June 2021, whereas biomass harvest occurred in late April 2022 (Table 2.3). In 2021, cover crop treatments that produced the most aboveground biomass were winter wheat, broadcast radish/ryegrass mix, drilled radish/ryegrass mix, and “Deer mix” (Table 2.5). Fallow with herbicide and “Graza” radish did not produce any cover crop biomass, while moderate amounts were produced by ryegrass alone, winter oat, spring oat, and forage collards. The low biomass results from radish and the winter and spring oats were likely due to winterkill after a cold winter in 2021, with December through February experiencing below average temperatures (Table 2.1). The fallow with herbicide treatment had the greatest weed density with 53 plants m^{-2} and was different than the lowest densities in winter wheat, “Deer mix”, and spring oat cover crop treatments, ranging from 16 to 22 plants m^{-2} (Table 2.5). More aboveground cover crop biomass was correlated with lower weed densities, as weed biomass data was not available in 2021 (Figure 2.4).

In 2022, winter wheat, drilled radish/ryegrass, ryegrass, and “Deer mix” cover crop treatments produced the greatest amount of aboveground cover crop biomass with none produced in the fallow with herbicide treatment. Results show that cover crop treatment was significant in terms of cover crop and weed biomass ($p < 0.05$), but not for weed densities (Table 2.5). In general, all cover crop treatments reduced weed biomass from 20% up to 99% compared to the fallow with herbicide treatment. Winter wheat, drilled radish/ryegrass mixture, and “Deer mix”

cover crops consistently produced the greatest biomass in both growing seasons (Figures 2.4 and 2.5). In contrast, broadcast radish/ryegrass mixture and ryegrass alone had very different amounts of biomass produced across years with 98% less in 2022 compared to 2021 (Table 2.5). This reduction in biomass could be attributed to a drier and colder than average winter and early spring for this area. November through February and April all had below average rainfall, and January through April experienced below average temperatures (Table 2.1). For example, January, February, and March were around 5 C cooler than average, and February and April received only 31% and 45% of the average precipitation, respectively. It is important to note that high weed counts within the quadrat did not necessarily correlate with high weed biomass amounts. For example, wheat had 238 weeds m⁻² that equaled 1.1 g m⁻² of weed biomass, indicating small weeds. This is corroborated by the findings of Hayden et al. (2012), who revealed that the cover crops used in their study reduced weed biomass per plant more than the weed density.

It is well documented that cover crop biomass is a generally reliable indicator of the level of weed suppression that can be expected, with higher amounts resulting in greater weed suppression (Baraibar et al. 2017, Cornelius & Bradley 2017, Hayden et al. 2012, Restuccia et al. 2020). Cornelius and Bradley (2017) found that oilseed radish and winter oat provided the least weed suppression due to their inability to overwinter in the experiment location (Central Missouri). The highest level of weed suppression from that study came from a cover crop mixture of cereal rye and hairy vetch, which resulted in a 68% to 72% reduction in weed biomass. Additionally, cover crop treatments of cereal rye and winter wheat reduced weed biomass by 53% and 50%. Hayden et al. (2012) revealed that cereal rye was the most effective cover crop treatment in suppressing weed biomass, with a 95% to 98% reduction in their

experiment. This study corroborates these findings of high weed suppression from cereals and cover crop mixtures, as the drilled radish/ryegrass mixture and the “Deer mix” mixture both reduced weed biomass by 98% compared to the fallow with herbicide treatment in 2022 (Table 2.5). Winter wheat and winter oat were very weed suppressive, both reducing weed biomass by 99% compared to the fallow with herbicide treatment. A study conducted by McLaren et al. (2019) also found that cover crop mixtures composed mostly or entirely of cereals produced more biomass, captured more resources, and suppressed more weed biomass than other cover crop treatments.

The previously mentioned cover crop treatments that reduced weed biomass the most, were also the highest biomass producers in this study (Table 2.5). Conversely, forage collards produced the lowest biomass across both years, and in 2022 reduced weed biomass by only 42% compared to the fallow with herbicide treatment. The fallow with herbicide treatment had the highest weed density in 2021 and the highest weed biomass in 2022 (Table 2.5). This is likely due to the herbicides used, which are both POST application herbicides with no residual activity in the soil. Thus, any weeds that emerged after herbicide application were not suppressed. Other studies have looked at the relative weed suppression of cover crops versus a fall application of residual herbicides and found that the weed control provided by the herbicides supersedes that of the cover crops (Cornelius and Bradley, 2017).

Conclusions

The purpose of this study was to evaluate the weed suppressive capabilities of several different cover crop monocultures and mixtures when seeded during the fallow period between corn and soybean in a conventional tillage crop rotation in southeast Kansas. In addition, weed emergence was monitored over the course of the fallow season to gain a better understanding of the timing and extent of weed emergence in this cropping system. The field management was designed to reflect that of a typical farmer in southeast Kansas so that the study has the potential for real world application for integrated weed control in agriculture. The inclusion of a fallow with herbicide control plot that had been treated with a fall application of POST herbicides was to compare relative levels of weed control between it and the cover crops. Overall, all cover crop treatments reduced weed biomass anywhere from 20% to 99% compared to the fallow with herbicide treatment. The winter wheat, winter oat, drilled radish/ryegrass mixture, and the “Deer mix” mixture were the most weed suppressive cover crop treatments, reducing weed biomass by 98% to 99% compared to the fallow with herbicide treatment. Weed densities were typically lower in all cover crop treatments compared to the fallow with herbicide control, however, this was an unreliable test of weed suppression, as some cover crop treatments such as winter wheat in 2022 had high weed densities, but very low weed biomass.

Weed species composition and emergence are highly variable between seasons due to changing environmental conditions. Four weed species were observed in both the weed rings and the 0.25 m⁻² quadrats in 2021 and 2022, including little barley, common chickweed, small-leaved bittercress, and blue mustard at varying proportions of the total and relative abundance. Purslane speedwell was observed in 2022, but not 2021, and small-leaved bittercress was observed in greater abundance in 2021 than in 2022. Temperature and soil water content are reported to be

two of the primary factors regulating seed germination (Baskin and Baskin 1988, Werle et al. 2012), but these factors were not tested in this study. However, weather data from Parsons, KS, revealed that the two winter seasons during this study were cooler and drier than average, potentially altering weed seed germination and emergence. Overall, the result of this research provides insight into the variability of winter annual weed germination and emergence in southeast Kansas under field conditions which can help producers predict the timing of emergence. Knowledge of the timing of emergence for certain weed species is helpful to producers in developing weed management strategies as part of an integrated weed management program. Additionally, the hypothesis that winter cereal cover crops and cover crop mixtures containing winter cereals are best for suppressing weeds was confirmed with the weed biomass results. Including aggressive grass species such as oats and wheat in cover crop mixtures can dramatically reduce the establishment and growth of weeds throughout the fallow period, thus reducing competition for early spring or summer cash crops and avoiding possible yield losses. The cover crops utilized in this study provided superior weed control relative to the fall application of herbicides. Ultimately, cover crops can be used as a component of multiple, complementary weed control tactics to manage weeds in cropping systems, reducing the reliance on synthetic herbicides that can result in widespread herbicide resistance.

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Figures and Tables

Table 2.1. Monthly rainfall totals (mm) and average monthly temperatures (°C) for the duration of the study and 30-year averages for Parsons, KS.

Month	Rainfall ^{ab}			Temperature ^{ab}		
	2020 - 2021	2021 - 2022	30-yr	2020 – 2021	2021 - 2022	30-yr
	average			average		
	mm			C		
August	37	88	83	24.4	26.1	32.2
September	105	80	119	20.0	23.1	27.2
October	109	104	98	12.5	16.2	21.1
November	86	13	75	10.2	8.6	13.9
December	40	31	52	3.5	8.4	6.7
January	98	1	36	2.2	0.8	5.6
February	9	14	45	-1.9	1.1	8.9
March	154	85	81	10.5	8.1	13.9
April	58	51	111	13.0	13.6	19.4
May	151	229	151	16.7	19.1	24.4

^a Weather data obtained from Kansas Mesonet weather monitoring station in Parsons, KS

^b 30-year average weather data obtained from US Climate Data website:

(<https://www.usclimatedata.com/climate/parsons/kansas/united-states/usks0459>)

Table 2.2. The composition, % weight, and seeding rates (kg ha⁻¹) of the cover crops seeded at Parsons, KS in both 2020 and 2021.

Cover crop treatment	Species composition	% of total weight	Seeding rate (kg ha ⁻¹)
Winter wheat	<i>Triticum aestivum</i> ‘Everest’	100	101
Radish	<i>Raphanus sativus</i> ‘Graza’	100	5.6
Spring oat	<i>Avena sativa</i>	100	67.2
Winter oat	<i>Avena sterilis</i>	100	67.2
Forage collards	<i>Brassica oleracea</i>	100	8
Drilled radish/ryegrass	<i>Raphanus sativus</i> / <i>Lolium perenne</i>	28 / 72	4.5 / 11.2
Broadcast radish/ryegrass	<i>Raphanus sativus</i> / <i>Lolium perenne</i>	23 / 77	5.6 / 19
Ryegrass	<i>Lolium perenne</i>	100	17.9
“Deer mix”	‘Gore’ Soft Red Beardless Winter Wheat ‘Hayden’ Spring Oat ‘Cosaque’ Black Oat ‘Elbon’ Cereal Rye ‘4010’ Spring Forage Pea ‘Kentucky Pride’ Crimson Clover ‘Frosty’ Berseem Clover ‘Mancan’ Buckwheat ‘Indian Head’ Spring Lentil Smart Radish Fixation Balansa Clover ‘Purple Top’ Turnip ‘Impact’ Forage Collards	21.4 17.1 14.3 14.3 14.3 4.3 4.3 2.9 2.9 1.4 1.4 0.7 0.7	56

Table 2.3. Dates of field operations and experiment procedures at the Parsons, KS.

Field Operation	Date of Operation		
	2020	2021	2022
Herbicide application	Oct 3	Oct 12	-
Cover crop seeding	Sep 28	Oct 6	-
P.V.C. rings installed	-	Mar 20/Oct 10	-
Soil sampling	-	-	Feb 14
Biomass sampling	-	Jun 6/7	Apr 28
P.V.C. ring removal	-	Jun 6/7	Apr 28
Cover crop termination	-	Jun 9	May 3

Table 2.4. Common names, Bayer code, % composition of total, and Relative Abundance (RA, %) of the winter annual weeds observed in the weed rings and the 0.25 m² quadrats throughout the 2020-2021 and 2021-2022 experimental years.

% of total is the total number of plants for a specific species, divided by the total of all species. Relative Abundance values were averaged over cover crop treatments.^{ab}

Year	Common Name	Bayer Code	% of Total		RA (%)	
			Weed rings	0.25-m ² quadrat	Weed rings	0.25-m ² quadrat
2020-2021	Little barley	HORPU	28.5	31.5	23.7 a	27.3 ab
	Common chickweed	STEME	24.5	36.4	23.9 a	33.2 a
	Small-leaved bittercress	CARPA	19.1	16.8	16.1 b	17.1 bc
	Henbit	LAMAM	17.8	-	20.1 ab	-
	Field pennycress	THLAR	6.7	8.1	8.2 c	12.6 c
	Blue mustard	COBTE	2.9	7.2	3.8 cd	9.8 c
	Mousetail	MYSMI	0.3	-	1.5 d	-
2021-2022	Little barley	HORPU	46.9	37.5	25.8 a	27.9 b
	Henbit	LAMAM	24.6	19.3	28.8 a	25.2 b
	Common chickweed	STEME	10.4	3.1	14.4 b	5.8 c
	Purslane speedwell	VERPG	10.0	38.9	13.5 b	37.2 a
	Small-leaved bittercress	CARPA	4.6	< 1	6.4 c	1.0 d
	Blue mustard	COBTE	2.5	< 1	5.8 c	0.3 d
	Field pennycress	THLAR	1.8	-	2.6 c	-
	Mousetail	MYSMI	-	< 1	-	1.3 d
	Horseweed	ERICA	-	< 1	-	0.3 d

^a Within each group and year, different letters indicate significant differences at a $p < 0.05$ level (Tukeys HSD).

^b Bayer codes retrieved from WSSA weed database.

Table 2.5. Average cover crop biomass, weed biomass (g m⁻²), and weed density (# plants m⁻²) for all cover crop treatments at time of termination in 2021 and 2022 at Parsons, KS.^{ab}

Year	Cover Crop	Cover Biomass	Weed Biomass	Weed Density
		g m ⁻²	g m ⁻²	# m ⁻²
2020-2021	Winter wheat	1103.1 a	-	20 a
	Broadcast radish/ryegrass	921.3 a	-	28 abc
	Drilled radish/ryegrass	876.8 a	-	29 abc
	“Deer mix”	823.8 ab	-	16 a
	Ryegrass	516.4 b	-	30 abc
	Winter oat	439.4 b	-	36 abc
	Spring oat	429.2 b	-	22 ab
	Forage collards	126.9 bc	-	47 bc
	Radish	0.0 c	-	40 abc
	Fallow with herbicide	0.0 c	-	53 c
2021-2022	Winter wheat	163.2 ab	1.1 a	238
	Broadcast radish/ryegrass	17.1 cd	91.5 bc	84
	Drilled radish/ryegrass	190.7 a	2.2 a	73
	“Deer mix”	154.5 ab	1.7 a	136
	Ryegrass	157.1 ab	18.0 ac	192
	Winter oat	110.2 bc	0.5 a	113
	Spring oat	85.3 bcd	25.3 ac	67
	Forage collards	1.8 d	74.0 bc	98
	Radish	41.7 cd	21.8 ac	107
	Fallow with herbicide	0.0 d	127.9 b	132

^a Within each group and year, different letters indicate significant differences at a p<0.05 level (Tukeys HSD).

^b No weed biomass collected in 2021.

Figure 2.1. Cumulative emergence (%) profiles for henbit (LAMAM) and common chickweed (STEME) from the 2021 to 2022 growing seasons.

Thermal Time (TT) accumulation begins on August 1, base germination threshold temperature for each species listed. Dotted line represents the first day of Fall, September 22, and the dashed line represents January 1. R-squared value represents how well the logistic model fits the observed data points.

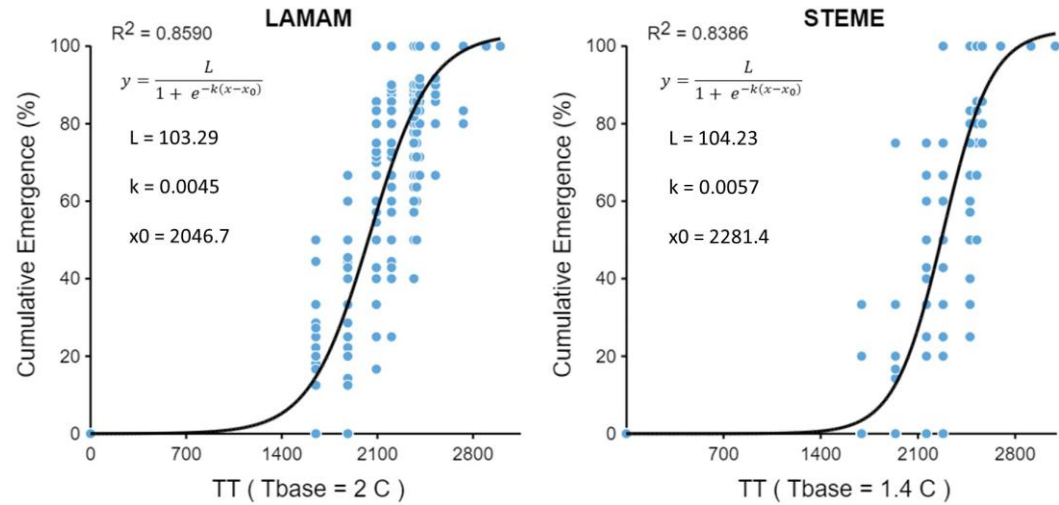
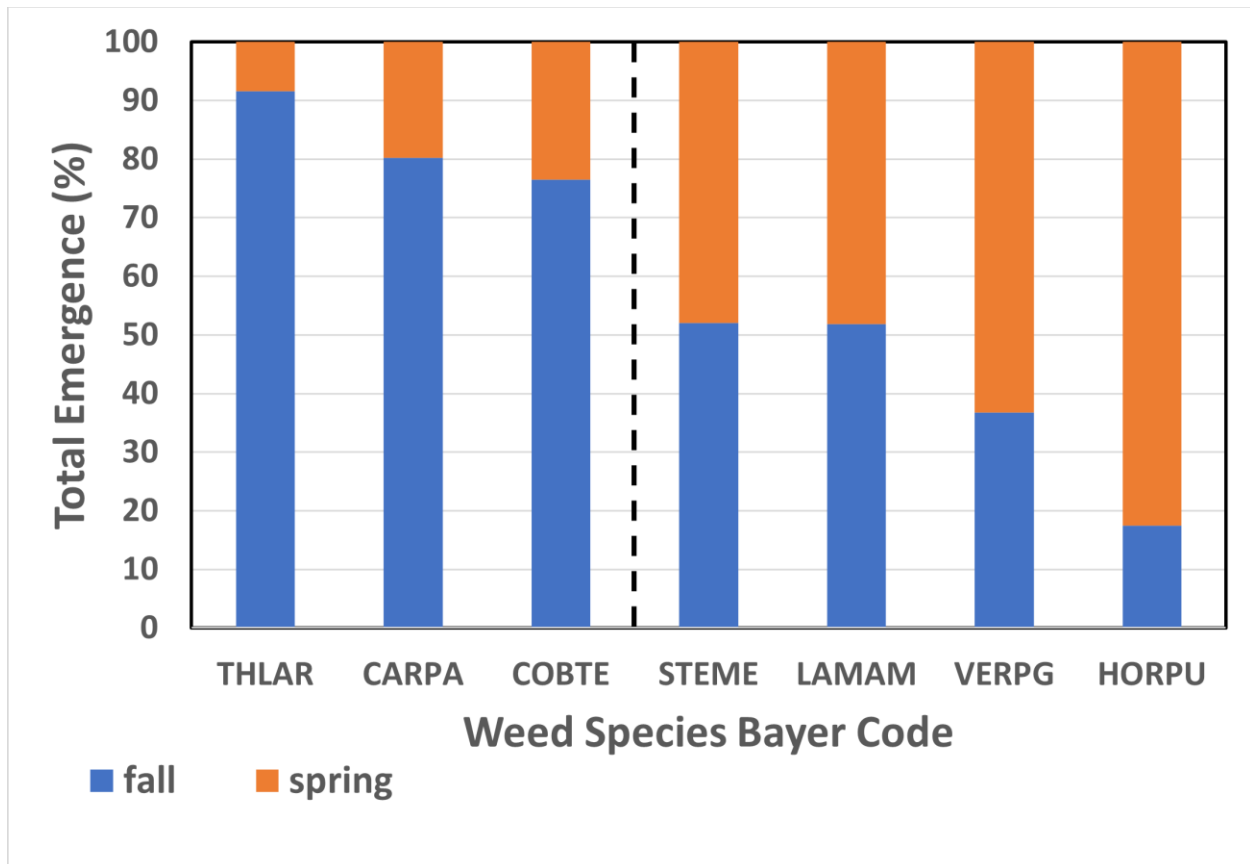


Figure 2.2. Total emergence (%) for each of the weed species from the 2021 to 2022 experimental year at Parsons, KS.

Dashed line separates the strict winter annual species to the left of the line, from the facultative winter annuals to the right of the line. Percent total emergence cutoff from strict versus facultative winter annuals used is 70% in the fall.^a



^a Bayer codes retrieved from WSSA weed database.

Figure 2.3. Total weed ring counts from the 2020 to 2021 and 2021 to 2022 seasons at Parsons, KS.

Total weed counts for the weed pull-rings averaged over all cover crop treatments and replications. Bars represent standard error (n=40). Significance symbol (Tukeys HSD) indicates differences at $p < 0.01$.

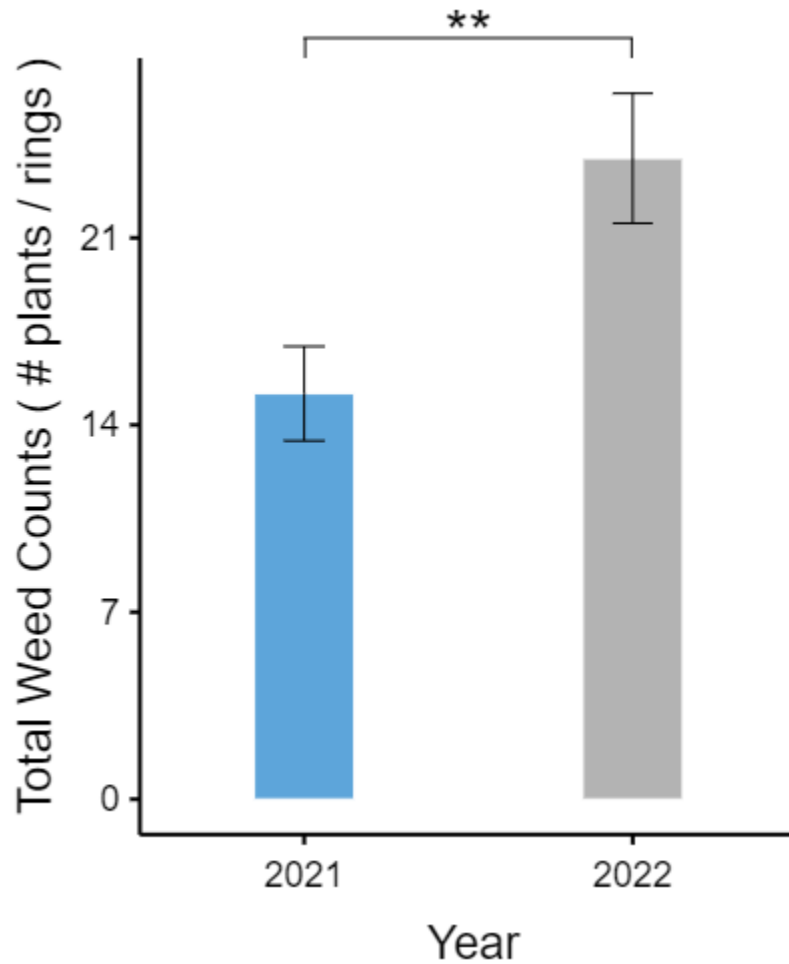
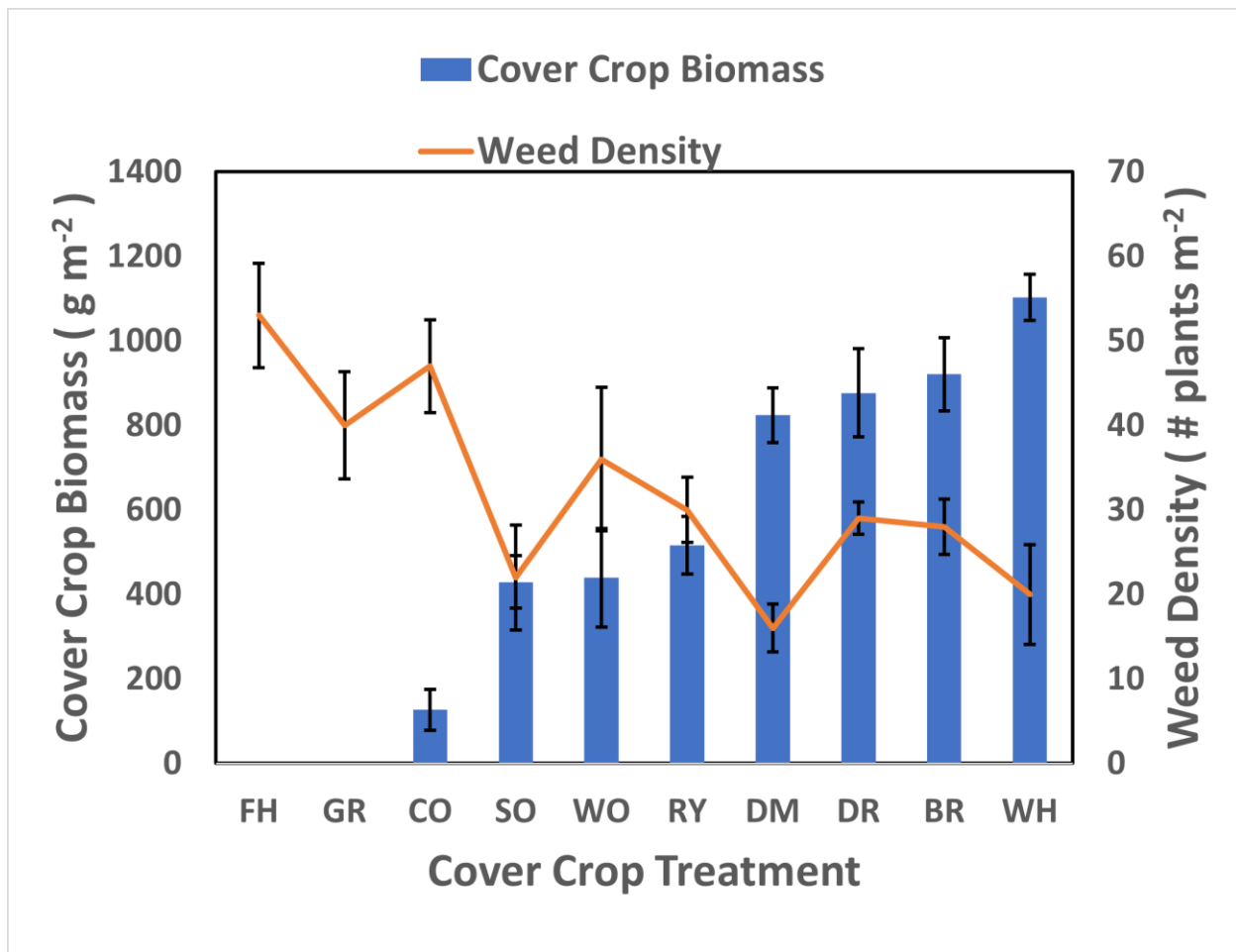


Figure 2.4. Mean cover crop biomass (g m^{-2}) and mean weed density ($\# \text{ plants m}^{-2}$) for each cover crop treatment in 2021 at Parsons, KS.

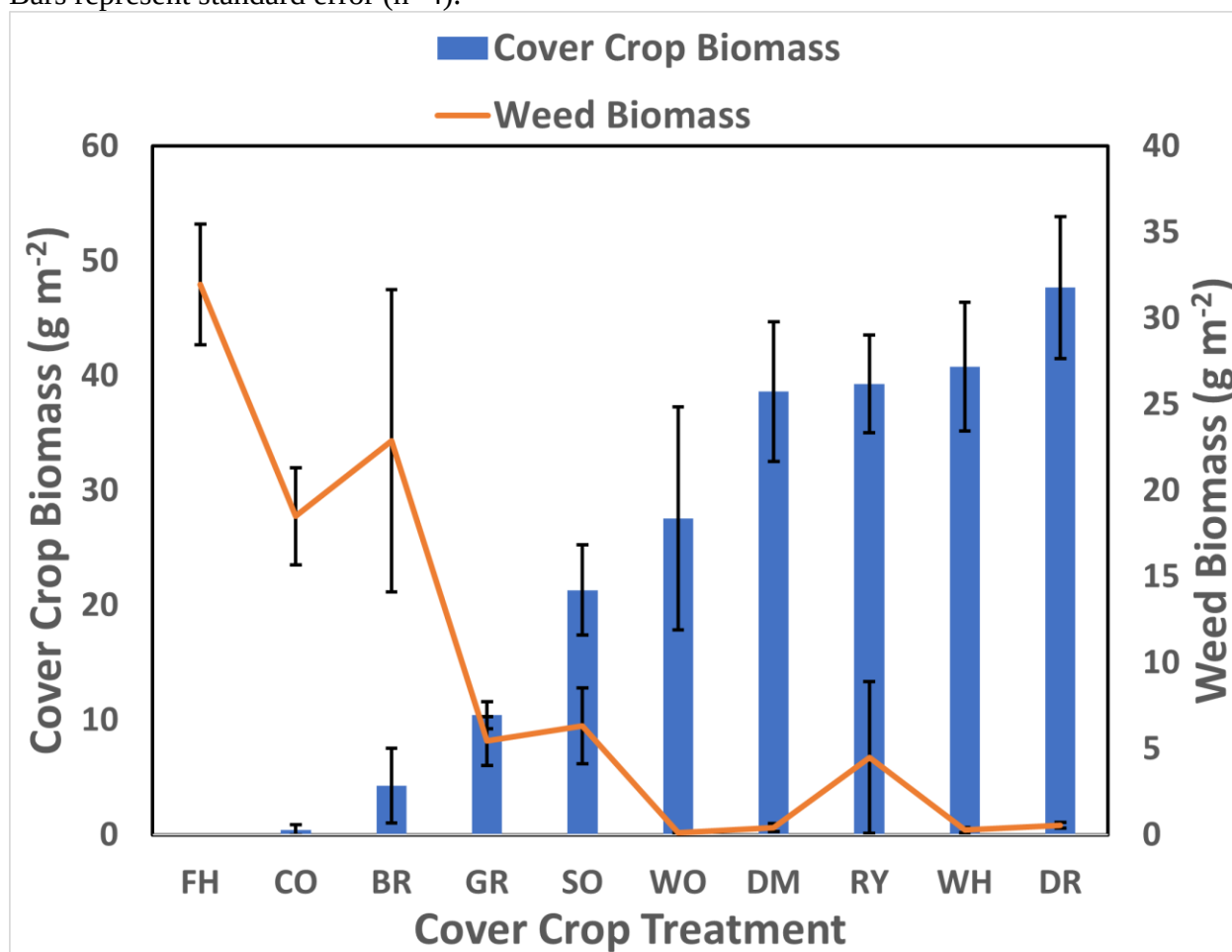
Bars represent standard error ($n=4$). ^a



^a Abbreviations: FH: Fallow with herbicide; GR: ‘Graza’ radish; CO: Forage collards; SO: Spring oat; WO: Winter oat; RY: Ryegrass; DM: “Deer mix”; DR: Drilled radish/ryegrass; BR: Broadcast radish/ryegrass; WH: ‘Everest’ wheat.

Figure 2.5. Mean cover crop biomass (g m^{-2}) and mean weed biomass (g m^{-2}) for each cover crop treatment in 2022 at Parsons, KS.

Bars represent standard error ($n=4$).^a



^a Abbreviations: FH: Fallow with herbicide; CO: Forage collards; BR: Broadcast radish/ryegrass; GR: ‘Graza’ radish; SO: Spring oat; WO: Winter oat; DM: “Deer mix”; RY: Ryegrass; WH: ‘Everest’ wheat; DR: Drilled radish/ryegrass.

Chapter 3 - Effects of Nitrogen and Sulfur Fertilizer on Cover Crop Weed Suppression and Allelopathic Potential

Abstract

Winter annual weeds have become more prolific in recent years with the increasing adoption of conservation tillage practices and the introduction of glyphosate resistant crops, resulting in reduced use tillage and residual herbicides. Understanding the timing and sequence of winter annual weed emergence under field conditions can be useful for producers to determine the best management practices to control these weeds. Cover crops planted over the fallow period have the potential to suppress weed germination and growth through direct competition and through the release of phytotoxic allelochemicals during residue decomposition. Allelochemical concentration within the plant depends on various factors, including the fertilization regime of the field. Field and laboratory experiments were conducted from fall 2020 to spring 2022 to document the emergence patterns of winter annual weeds and evaluate the effects of four different winter cereal cover crops and three different fertility regimes on weed suppression. Fall-seeded cover crops were “Elbon” and “Rymin” rye (*Secale cereale* L.), “Everest” winter wheat (*Triticum aestivum* L.), and “Surge” triticale (*Triticale hexaploide* L.). Weed species and their emergence were recorded within permanent P.V.C. rings from the time of cover crop planting until termination in late spring of each year. Three fertility treatments, a no fertilizer control, nitrogen fertilizer, and nitrogen plus sulfur fertilizer, were broadcast applied in the spring of each year. Cover crop biomass and weed densities and biomass were collected within 0.25 m² quadrats in late spring before cover crop termination. All cover crops reduced weed

biomass anywhere from 89% up to 98% compared to the fallow control, with “Elbon” rye having the lowest weed biomass observed. Nitrogen and nitrogen plus sulfur fertilizer treatments increased both cover crop and weed biomass relative to the no fertilizer control. A portion of the cover crop biomass was used in a Palmer amaranth (*Amaranthus palmeri* L.) seed germination bioassay to evaluate allelopathy potential in summer 2021 and 2022. Extract concentrations of one, three, and five % were prepared using the dried cover crop biomass from that year and applied to petri dishes containing 25 Palmer amaranth seeds and placed in a growth chamber for 96 hours at 29 ± 4 C with a 16-hour photoperiod. Increasing extract concentration decreased weed seed germination with 5% resulting in the lowest weed seed germination in both years. Fertilizer treatment had variable effects across years. In 2022, the nitrogen fertilizer treatment reducing weed seed germination the most relative to the no fertilizer and nitrogen plus sulfur fertilizer treatments. Further research will be necessary to investigate the effect of fertility regimes on weed suppression with cover crops.

Introduction

The use of cereal rye (*Secale cereale* L.) as a cover crop has become more common in recent decades for its aggressive growth habit, high biomass production, and reported allelopathic potential in various cropping systems, including corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean (*Glycine max* (L.) Merr.). Allelopathy is the phenomenon by which plants can suppress the germination or growth of surrounding plants through the production and release of phytotoxic compounds known as allelochemicals (Flood and Entz, 2009). Winter wheat (*Triticum aestivum* L.) and triticale (*Triticale hexaploide* L.) have also been explored for their allelopathic potential, which could be used in weed management (Flood and Entz 2009, Lam et al. 2012, Petcu et al. 2017). Weeds are arguably the most important biotic constraints in agricultural systems, reducing crop yield by up to 100% if left uncontrolled (Chauhan 2020). In addition, winter annual weeds can be problematic to producers by forming dense mats of vegetation that delay the soil warming, inhibit field operations such as spring tillage and planting, and compete with spring planted cash crops for limiting resources (Werle et al. 2014, Woolam et al. 2018). Weed control is particularly challenging in reduced or no-tillage systems with little or no synthetic herbicide use (Shultz et al. 2013) and replacing synthetic herbicides with other direct methods often results in inadequate weed control. Thus, allelopathic weed control by cover crops is a desirable alternative method and can be a component of an integrated weed management (IWM) program.

Cover crops are often planted over the winter fallow season in between cash crops primarily to reduce soil erosion and increase the quality of the soil and surrounding water sources through runoff reduction (Dabney et al. 2001, Price et al. 2005). In addition, cover crops can compete with weeds for limiting resources such as water, nutrients, and light, thus assisting

in the control of weeds and reducing herbicide inputs and weed control costs (Dabney et al. 2001). Weed suppressive cover crop stands can improve the long-term weed population management by reducing weed seed production and subsequent population growth, thereby decreasing competitive pressure on cash crops (Baraibar et al. 2017). They are particularly important in organic systems, such as reduced tillage or no-tillage, where certain weeds such as giant foxtail (*Setaria faberi* Herrm.) and redroot pigweed (*Amaranthus retroflexus* L.) can become more difficult to control compared to conventionally tilled fields (Buhler and Oplinger 1990). In the United States, soybean conservation tillage systems are around 50% of the total soybean hectares, and up to 97% of those are sprayed with synthetic herbicides (Price et al. 2005). Reducing the use of synthetic herbicides in these systems is not only economical and better for the environment, but also reduces the risk of weeds developing herbicide resistance, which can make them difficult to control (Heap 2020).

Cover crop residues left on the soil surface can modify the germination of weed seeds by decreasing light availability, lowering soil temperature, and through the release of phytotoxic chemicals that inhibit weed seed germination (Creamer et al. 2021). These phytotoxins are known as allelochemicals, which are the primary compounds that cause the inhibition of weed germination and growth, also known as allelopathy. For example, cereal rye residues on the soil surface can reduce the emergence of common summer annual weeds such as common ragweed (*Ambrosia artemisiifolia* L.), green foxtail (*Setaria viridis* (L.) P. Beauv.), redroot pigweed, and common purslane (*Portulaca oleracea* L.) by 43%, 80%, 95%, and 100% respectively (Creamer et al. 2021). Barnes and Putnam (1983) found that the residues of fall-planted and spring-killed cereal rye reduced total weed biomass by 63% compared to a control of poplar (*Populus excelsior* L.) mulch. Cover crop residues are still effective if incorporated into the soil through

tillage, as a study by Al-Khatib et al. (1997) showed that fall planted rapeseed (*Brassica napus* L.) incorporated into the soil in the spring reduced weed density by 73 to 85% and weed biomass from 50 to 96%.

The dominant allelochemicals found in winter cereals such as rye, wheat, and triticale, are phytotoxic cyclic hydroxamic acids, known as benzoxazinones (BX's) (Macias et al. 2005). BX's exist as stable, nontoxic glucosides in the vacuole of plant cells that are released as phytotoxic degradation products through enzymatic hydrolysis upon tissue wounding and decay (Burgos et al. 1999). BX's can also be released through living plant tissue by root and shoot exudation, although in lesser quantities than during residue degradation (Tabaglio et al. 2013). In fact, the slow release of allelochemicals from cover crop residues as they degrade over time can result in weed control up to four to eight weeks after mulching (Schultz et al. 2013). The concentration of BX's in the tissues of winter cereal cover crops depends on many factors, including the cover crop genotype, the age of the plant at termination, fertilization regimes, and various environmental factors such as temperature, water supply, and light intensity. Plant stress has been reported to enhance BX production and induce shifts in the organ specific production of these compounds, as defoliation increased BX synthesis and changed the allocation from the shoots to the roots, where they were released as exudates into the soil (La Hovary 2011).

The susceptibility of weed or crop species to various allelochemicals is often influenced by seed size, and studies have shown that the seedlings of certain large-seeded crop species are more tolerant of stress due to larger reserves within the seed for respiration (Leibman & Sundberg 2006). The implication of this is that cover crops can potentially be utilized as a component of weed management systems that could suppress weeds while leaving crops unaffected. For example, Leibman and Sundberg (2006) found that cover crop residues inhibited

the germination and growth of small-seeded weeds such as large crabgrass (*Digitaria sanguinalis* (L.) Scop.), goosegrass (*Eleusine indica* (L.) Gaertn), and prickly sida (*Sida spinosa* L.), while increasing the germination and growth of large-seeded crops such as snap bean (*Phaseolus vulgaris* L.). Burgos and Talbert (2000) observed that certain large-seeded crops such as corn and several varieties of melon (*Cucurbita* spp.) were more tolerant of allelochemicals than small-seeded plant species such as Palmer amaranth. As previously mentioned, the production of BX's varies between the cultivars of the same species and certain varieties of cereal rye such as "Bonel" and "Aroostok" have particularly high levels of BX's per gram of plant tissue (Burgos et al. 2000). However, there is an apparent downside to high allelopathic potential, as "Bonel" and "Aroostok" cereal rye also produced the least amount of biomass of the cultivars tested. Petcu et al. (2017) assessed the allelopathic potential of 24 different varieties of winter wheat and triticale by incorporating the cover crop residues into soil growing redroot pigweed and by conducting a seed germination bioassay. Overall, they found varying levels of germination and growth inhibition, with six of the 24 cultivars inhibiting redroot pigweed root elongation by more than 40%. Termination timing is also important, as Burgos et al. (1999) found that the total concentration of allelochemicals from shoot tissues of greenhouse grown "Bates" cereal rye, increased between 30 and 60 days after planting and declined thereafter. In general, known allelopathic cover crop varieties can be utilized as an alternate weed management tool to reduce weed populations.

Stress factors such as low soil fertility have been reported to enhance the allelochemical content in cereal rye tissues (Schultz et al. 2013) and various studies have examined the effect of nitrogen fertilizer on the allelochemical content of cereal rye (Gavazzi et al. 2010, Mwaja et al. 1995, Reburg-Horton 2005, Tabaglio et al. 2013). Weed seedling bioassays are often conducted

to measure the allelopathic potency of cover crop residues, due to the difficulty of separating effects of physical and chemical effects on weed suppression in field experiments (Flood and Entz 2009). Moreover, seed germination bioassays are often essential in screening certain crops for allelopathic potential (Petcu et al. 2017). Studies have shown that higher nitrogen fertilizer rates increase the allelopathic potential of rye (Gavazzi et al. 2010, Tabaglio et al. 2013) measured as total allelochemical content per gram of shoot tissue. Other studies have shown that allelochemical content was higher in the low rates or absence of nitrogen fertilizer treatments (Reburg-Horton 2005, Mwaja et al. 1995). Thus, a range of results that have come from experiments investigating the effects of nitrogen fertilizer on cereal rye allelopathic potential. There is little research on the effects of sulfur fertilizers on cover crop allelopathic potential, however, studies have shown that sulfur can increase the nitrogen use efficiency of various winter cereal cover crops, resulting in higher shoot biomass among other effects (Carciochi et al. 2017, Salvagiotti et al. 2009). The increase in aboveground biomass may improve the cover crop's physical suppression of weeds, but unless BX synthesis increases within the plant, it may dilute the concentration of BX's per gram of plant tissue. Additionally, since BX production has been reported to increase under stress conditions, including low soil fertility, the addition of nitrogen and sulfur fertilizers may decrease the allelopathic potential of winter cereal cover crops.

Therefore, one of the objectives of this study was to test the effects of nitrogen and sulfur fertilizers on winter cereal cover crops allelopathic potential through a weed seed germination bioassay. The hypothesis of this experiment was that the increase in soil fertility would decrease the allelopathic potential of the cover crops, resulting in an increased germination rate of weed seeds with the addition of nitrogen and sulfur fertilizers. More information on the effects of soil

fertility on the allelopathic potential of winter cereal mulch may be useful for producers deciding whether or not to include cover crops as part of a sustainable weed management program, thus reducing the amount of synthetic herbicides used. A second objective was to document the emergence of winter annual weeds in the field to determine the extent and timing of their emergence in winter cereal cover crops through the fallow period. Modelling the emergence of winter annual weeds using the accumulation of soil thermal time units may be useful in developing weed emergence profiles, as soil temperature has been reported as being one of the primary factors driving seed germination (Baskin and Baskin 1988). There is limited information on winter annual weed emergence in Kansas, and for farmers to better manage weeds in their fields, the timing of weed emergence is critical.

Materials and Methods

3.1: Field Descriptions and Experimental Design

Field experiments were conducted to determine the emergence patterns of winter annual weeds in different cereal cover crops and the effect of various fertility regimes on cover crop allelopathic potential during two experimental years (Fall 2020 to Spring 2021, Fall 2021 to Spring 2022) in Riley County at the USDA Plant Materials Center (PMC) near Manhattan, Kansas (39°08'36.56"N, 96°38'15.01"W, 314.25 m). The monthly average rainfall and temperatures are presented in Table 3.1. The soil type is a Bellevue silt loam (mesic Fluventic Haplodoll) with 2.6% organic matter, a pH of 5.8, 23.9 ppm NO_3^- -N, and 2.4 ppm SO_4^{2-} -S in 2020, and 1.0% organic matter, a pH of 6.4, 1.9 ppm NO_3^- -N, and 0.4 ppm SO_4^{2-} -S in 2021. Treatment factors were five different cover crop treatments, triticale “Surge”, winter wheat “Everest”, two cereal rye varieties “Elbon” and “Rymin”, and a fallow control, and three fertility regimes (none, nitrogen only, nitrogen+sulfur). Neighboring field sites were used over the two years. In 2020 to 2021, the experimental design was a split-plot RCBD with four replications while in 2021 to 2022, the experimental design was an RCBD with a factorial arrangement of five cover crop treatments and three fertility regimes with four replications. From 2020 to 2021 the total size of the experimental area was 9.75 m by 28.34 m, with cover crop variety as the whole plot factor (1.95 m by 7.08 m), and fertilizer as the sub-plot factor (1.95 m by 2.36 m). From 2021 to 2022 the experimental area measured 25 m by 28 m with individual plots measuring 2 m by 7 m with cover crop treatment and fertilizer treatment as the whole plot factors.

The plots were in a no-till field with no herbicide applied that was previously harvested for soybean. Four winter annual cover crops were planted on September 24, 2020 and September

28, 2021, with a no-till drill at a depth of 2.54 cm: triticale “Surge” and winter wheat “Everest” at 100 kg ha⁻¹, and “Elbon” and “Rymin” cereal rye at 112 kg ha⁻¹. Within two weeks of the cover crops being planted, two permanent P.V.C. rings (d = 20.32 cm) placed within each cover crop plot to document weed emergence. Within one of the rings, weed species were identified, enumerated, and removed by hand on a bi-weekly basis until cover crop biomass collection the following spring (Table 3.2). Emerged seedlings were identified and pulled when the cotyledons were fully expanded to ensure accurate identification of the seedlings and minimal soil disturbance. The other ring was used for comparison as the weeds were identified and enumerated, but not removed.

3.2. Fertilizer Description and Cover Crop Termination

The fertilizer treatments consisted of a zero-fertilizer control (0), a nitrogen fertilizer application (N), and a nitrogen and sulfur fertilizer combination (N+S). Fertilizer rates were calculated using the Kansas State University winter wheat recommendation of 30 pounds nitrogen per acre, and 13 pounds of sulfur per acre. Urea (46-0-0) was applied at 73 kg ha⁻¹ and elemental sulfur (0-0-0-90 s) was applied at 16 kg ha⁻¹ in 2021. In 2022, urea was applied at 45 kg ha⁻¹ and ammonium sulfate (21-0-0-24 s) at 60 kg ha⁻¹. This was intended to simulate a situation in which a producer top-dresses fertilizer for a winter wheat crop in the spring and to observe how it affects weed and cover crop growth. Each plot received a broadcast application of the appropriate fertilizer treatment on April 5, 2021 and March 4 2022 (Table 3.2). In 2021, the cover crops were terminated two weeks before soybean planting on May 12, using a forage harvester with a cutting swathe of 0.91 m. To determine cover crop biomass, a random sample of clippings from the forage harvester were collected by hand from each plot and placed into pre-weighed small (9 ± 0.5 g) and large brown paper bags (15 ± 0.7 g). In 2022, two cover crop

biomass samples were collected per plot using a 0.25 m² quadrat. The cover crops were clipped at ground level and placed into a large paper bag (~15 g), and weeds were individually counted and placed in a small paper bag in 2022 (~9 g). Weed biomass or weed densities were not collected in 2021 but determined in 2022. All cover crop samples were weighed to measure wet biomass (g) and then put into a drying oven for 96 hours at 60°C. The bags were re-weighed to measure dry biomass (g), and percent dry biomass was calculated based on the difference.

3.3. Laboratory Bioassay Experiments

Laboratory experiments took place during the summers of 2021 and 2022 at the Kansas State University Department of Agronomy weed ecology lab using the dried above ground cover crop residues from each year from the two field studies collected at the USDA PMC (2020-2021, 2021-2022). Dried shoot biomass from each cover crop treatment and each experimental year were ground separately in a coffee grinder. Powdered shoot material contained all aboveground parts of the cover crops including florets and were stored in individually labeled plastic sandwich bags, weighed, and set aside for use in extract preparation. Samples of the powdered material from each year were sent to the Kansas State University plant analysis lab to test for total nitrogen (%), total carbon (%), phosphorus (%), and potassium (%). For each treatment, 1%, 3%, and 5% extract solutions were made using 1g, 3g, and 5g of the powdered residues. The powdered residues were placed in a 10 by 10 mm section of cheesecloth and tied at the top with twine to form a pouch. The pouches were then placed in 250 mL containers containing 100 mL of tap water, agitated with a glass stirring rod and then stored in a refrigerator at 0° C for 24 hours until use. Four mL of each extract concentration (1%, 3%, 5%) from each treatment were added to 95 x 15 mm plastic petri dishes lined with two filter papers (Whatman No. 1), 25 Palmer amaranth (*Amaranthus palmeri* L.) seed and an additional filter paper on top of the seeds.

The control consisted of four mL of tap water. The dishes were closed, sealed with Parafilm, and placed in a growth chamber with a 16-hr photoperiod at 29 ± 4 C. Petri dishes were staggered by replication in the growth chamber, for example, all three replications of several cover crop and fertilizer treatments were placed in the growth chamber while the rest were being prepared. Germination was determined after 96 hr of incubation by the emergence of a visible radicle from the seedcoat. The experimental design was an RCBD with a factorial arrangement of four cover crop treatments, three fertility regimes, and three extract concentrations with one replication per field plot in 2021, and three replications per field plot in 2022. Each year included 15 replications as no extract controls with tap water.

3.4. Weed Flora and Emergence

Patterns of weed emergence were determined based on the accumulation of thermal time units. Data for the daily maximum and minimum air temperatures, precipitation, and 5 cm depth soil temperature were obtained from the nearest weather station in Ashland Bottoms, KS (Kansas Mesonet; site ID: ASBK1; 39.126° N, 96.637° W). Weed emergence data were converted from bi-weekly counts to cumulative counts, and then to cumulative emergence (%) based on the total plant emergence per ring during that experimental year (2020-2021, 2021-2022). Cumulative emergence curves were constructed for each weed species per year using the 5 cm soil thermal time units. For winter annual weeds, cumulative soil thermal time was calculated for each day after August 1 of each year the experiment was conducted. August 1 was chosen as the start date of accumulation of soil TT units based on previous studies and the fact that winter annual weeds are released from dormancy by high summer temperatures and germinate when temperatures start to gradually decrease (Baskin & Baskin, 1988). Cumulative soil TT was calculated as:

$$TT = \sum_{i=1}^n (T_{mean} - T_{base})$$

where: T_{mean} = daily mean soil temperature at 5 cm depth (C); T_{base} = base soil temperature for each species for seedling emergence (C).

The base temperatures for germination used in the equation were 0 C for horseweed (*Erigeron canadensis* L.), carpetweed (*Mollugo verticillata* L.), and flixweed (*Descurainia sophia* (L.) Webb ex Prantl), 1.4 C for mouseear chickweed (*Cerastium fontanum* Baumg. ssp. *vulgare* (Hartm.) Greuter & Burdet), 1.7 C for field pansy (*Viola bicolor* Pursh.), and 2 C for henbit (*Lamium amplexicaule* L.) (Werle, 2012). Weed counts were modelled using a logistic function to describe cumulative emergence by species.

$$y = \frac{L}{1 + e^{-k(x-x_0)}}$$

where: y is cumulative emergence (%) as a function of cumulative soil TT (C), x_0 equals cumulative soil TT value of the logistic function midpoint, L is the maximum cumulative emergence, and k is the logistic growth rate or steepness of the curve (Saini 2021).

Each individual weed species was grouped into strict winter annuals versus facultative winter annuals by summing the number of emerged seedlings from the weed pull rings before and after January 1. These weed count sums were then converted into a percent of total before and after January 1. If the majority (>70%) of the seedlings emerged before January 1, then they were classified as mostly fall-emerging or strict winter annuals. If the proportion of seedlings emerged before and after January 1 was nearly even (50:50), then they were classified as fall and spring emerging winter annual weeds. If the majority (>70%) of seedlings emerged after January

1, they were classified as facultative winter annual weeds (Baskin and Baskin 1988, Cici and Van Acker 2011).

Analysis of weed flora and diversity were determined by calculating the proportion of each weed species as a percentage of the total counts for both the weed pull ring counts and the quadrat counts taken at cover crop termination. The numeric abundance and distribution of individual weed species in the weed flora of the plots were calculated using four quantitative measures adapted from Thomas (1985): Frequency (F), Relative Frequency (RF), Relative Density (RD), and Relative Abundance (RA).

Frequency value is the presence (1) or absence (0) of species k in each weed pull ring.

Relative Frequency for species k in each weed pull-ring or 0.25-m² quadrat (RF_k) =

$$\frac{\text{Frequency value of species } k}{\text{Sum of frequency values for all species}} * 100$$

Relative Density for species k in each weed pull-ring or 0.25-m² quadrat (RD_k) =

$$\frac{\text{Total counts for species } k}{\text{Sum of counts for all species}} * 100$$

Relative Abundance for species k in each weed pull-ring or 0.25-m² quadrat (RA_k) =

$$\frac{RD_k + RF_k}{2}$$

3.5. Data Analysis

All data were analyzed using the PROC GLIMMIX procedure in SAS (v. 9.4, SAS Institute, Cary NC). The models were checked for normal distribution and equal variances and pairwise comparisons within fixed effects observed with the LSMEANS statement. Weed count

and relative abundance data were analyzed by weed species and cover crop treatments, including all interactions. Relative abundance values were calculated for both the ring weed counts and the 0.25-m² quadrat weed counts. If a weed species appeared in one year and not the other, it was omitted from the ANOVA analysis between years in order to get a clear comparison. Biomass data were analyzed separately by either cover crop or weed biomass with cover crop treatment and year, including all interactions. Weed biomass and weed density by cover crop treatment was not collected in 2021, thus only cover crop biomass was analyzed. If the two- or three-way interactions were not significant at $P < 0.05$, further analyses were performed for two-way interactions and/or fixed effects. Cover crop was considered a fixed effect, while year and replication were considered random effects. The weed counts were modelled using a logistic function to describe cumulative emergence by species over cumulative soil TT. Bioassay germination (%) data were analyzed by cover crop, fertilizer, and extract concentration, including all interactions as fixed effects. Replication was considered a random effect. Bioassay results were modelled with simple linear regression to describe germination (%) over extract concentration. Logistic model and linear regression models, function parameters, and emergence graphs generated by GraphRobot (Wang 2019).

Results and Discussion

3.1. Weed Species Composition and Emergence

Throughout both experimental years, a total of seven weed species with varying levels of relative abundance were observed in the weed pull rings (Table 3.3). Only one of them, henbit, occurred in both years. Horseweed was observed in the 2020 to 2021 experimental year, whereas mouseear chickweed, field pansy, flixweed, and carpetweed were observed during the 2021 to 2022 experimental year. Analyses of the relative abundance was performed within years, separately. A two-way ANOVA with weed species and cover crop treatment as the two factors revealed that there was no significant interaction for 2020 to 2021. Weed species differed but cover crop did not. Henbit was the dominant weed, accounting for 92% of the total composition and a relative abundance of 73.3, with horseweed accounting for the remaining 8% with a relative abundance of 26.7 (Table 3.3). In the second year (2021 to 2022) cover crop treatment and weed species interacted to affect relative abundance, with the control plus nitrogen cover crop treatment having greater weed RA than all other cover crop treatments. Henbit was 83% of the total composition with a relative abundance of 54.3, with mouseear chickweed, field pansy, flixweed, and carpetweed ranging from 9% to 1% and relative abundance ranging from 17.7 to 4.3. In both years, henbit was the dominant weed species, accounting for over 80% both years.

By the time of cover crop termination in 2022, quadrat weed counts consisted of a total of eight weed species, with six of them being the same as the weed pull ring species, including henbit, mouseear chickweed, field pansy, flixweed, and carpetweed (Table 3.3). The two additional species were dandelion (*Taraxacum officinale* F.H. Wigg.) and blue mustard (*Chorispora tenella* (Pall) DC.). A two-way ANOVA showed that the interaction of cover crop treatment and weed species was not significant. Quadrat weed counts did not differ by cover crop

treatment, but they did differ by weed species. Henbit was most of the total composition, accounting for 58% and a relative abundance of 46.5. Mouseear chickweed, field pansy, and carpetweed were the next most abundant weeds, all around 10% of the total composition and relative abundance from 1.77 to 4.3. The rest of the weed species ranged in total composition from 5% to less than 1%, and relative abundance ranging from 3 to 1.4 (Table 3.3). There were more weed species observed in the 0.25 m² quadrats than in the weed rings, possibly due to the larger sampling area of the quadrats versus the weed pull rings.

Throughout both experimental years, henbit emergence was distinctly concentrated in the fall, with 85% of the total seedlings germinating before January 1 (Figures 3.1 and 3.2). Most of the henbit seedlings emerged between September 22 and January 1, and had a high maximum cumulative emergence, indicating that it was present in most of the cover crop treatments. Horseweed was only seen in the rings in 2021, but its emergence was evenly split with 50% of the seedlings emerging in the fall and 50% emerging in the spring. Comparing the rate of cumulative emergence of the logistic curve between henbit and horseweed, horseweed seedlings emerged much more quickly than henbit ($k=0.012$ vs. $k=0.0055$). Mouseear chickweed was another mostly fall germinator, with 73% of its seedlings appearing in the fall versus 27% in the spring. Mouseear chickweed had a very quick germination period ($k=0.01$) and had a high maximum cumulative emergence (Figure 3.2). Henbit was a prominent weed in 2022 and had a much faster emergence rate compared to 2021 ($k=0.008$). The remaining four weeds, flixweed, field pansy, and carpetweed were all mostly spring germinators, with 69%, 84%, and 100% emerging in the spring, respectively (Figure 3.3).

The henbit emergence results are corroborated by studies in Louisiana (Woolam et al. 2019) and Nebraska (Werle et al. 2014). In these studies, henbit behaved as a strict winter

annual, with over 80% of the seedlings emerging in the fall. Baskin and Baskin (1988) found that henbit behaves as a mostly fall germinating weed species assuming the seeds undergo afterripening requirements of high summer temperatures (25/15 C and 20/10 C), which breaks their dormancy. Horseweed seeds are nondormant after maturing in late summer and early fall, thus can germinate immediately if environmental conditions such as soil water content and temperature are within the appropriate range (Baskin and Baskin 1988). Late-dispersing seeds from horseweed can germinate in the spring, thus half of the horseweed germinating in the fall and the other half germinating in the spring is to be expected. Werle et al. (2014) found that flaxweed and field pansy seedlings emerged mostly in the fall in Nebraska, similar to henbit. In this study, most of the flaxweed and field pansy seedlings emerged in the spring, opposite to the findings of Werle et al. (2014). Temperature and its influence on seed dormancy and germination has been reported as being the primary factor regulating germination, with soil moisture content and light availability as secondary factors (Baskin and Baskin 1988, Werle 2012). Temperatures were near the 30-year average during the winter of the second year (2021 to 2022), thus it is possible that fluctuating temperatures over the winter and early spring induced spring germination of field pansy and flaxweed from the seedbank (Benech-Arnold et al. 2000). The variability in the weed species composition between years may be due to differences in the weed seedbank as neighboring fields were used. There were four species observed in the 2021 to 2022 year that were not observed the previous year. The weed seedbank can be a primary source of weed species composition within a location (Travlos et al. 2021). In general, seasonal environmental patterns and how it relates to weed emergence are often site-specific, thus emergence data are useful in the region where the experiments were conducted.

3.2. Cover Crop Treatments and Weed Suppression

Total weed counts from within the pull rings from the first year (2020 to 2021) did not differ between cover crop treatments. The pull ring weed counts from the second year (2021 to 2022) did not result in an interaction between cover crop treatment and fertilizer treatment. Neither cover crop treatment nor fertilizer treatment were significant factors as far as pull ring weed counts, however, the individual weed species counts differed from one another. No differences in cover crop biomass were found between cover crop treatment or fertilizer treatment in 2021 because fertilizers were applied late in the season (April 5, 2021) and crops did not have time to respond before termination. Additionally, elemental sulfur was used as the sulfur source that year, which is not available for plant uptake until oxidation to sulfate occurs. The subplot size was also small (2m by 2.4m), so fertilizer influence was not likely restricted to the subplots, thus diluting the effects of the fertilizer treatments. In the second year, there was no interaction between cover crop and fertilizer treatments, but main effects were significant. The average cover crop biomass ranked in order from highest to lowest were ‘Elbon’ rye, ‘Rymin’ rye, triticale, and winter wheat, with ‘Elbon’ rye differing from triticale and winter wheat ($P < 0.05$) (Table 3.4). Across all the cover crop treatments, the nitrogen fertilizer treatment did not increase cover crop biomass significantly relative to the control, but the nitrogen plus sulfur fertilizer treatment did (Table 3.4). Overall, the nitrogen fertilizer treatment resulted in a 2.5% increase in biomass compared to the no fertilizer treatment, and the nitrogen and sulfur fertilizer treatment increased biomass by 64% compared to the nitrogen treatment. Tabaglio et al. (2013) found that ‘Primizia’ rye seeded in plots that had been fertilized the previous spring for corn with nitrogen at rates of 250 and 300 kg ha⁻¹ increased in biomass from 81% to 135% compared to a

no fertilizer control. The significant increase in biomass between the nitrogen treatment and the nitrogen and sulfur treatment could be due to improved nitrogen use efficiency within the cover crops (Salvagiotti et al. 2009). With winter wheat, the addition of sulfur fertilizer at 30 kg ha⁻¹ increased the plant's nitrogen recovery efficiency from the soil at all the tested nitrogen rates (26 to 104 kg ha⁻¹), resulting in increased grain yield (Salvagiotti et al. 2009). Additionally, Carciochi et al. (2017) found that winter wheat had a 20% increase in shoot biomass relative to sulfur deficient winter wheat, indicating the synergistic effects of sulfur and nitrogen on biomass production.

An analysis of the weed biomass revealed similar results to the cover crop biomass, with main effects of cover crop and fertilizer being significant. All cover crop treatments reduced weed biomass significantly compared to the no cover check treatment, anywhere from 89% to 98% (Table 3.4). 'Elbon' rye reduced weed biomass the most, by 98%, whereas winter wheat reduced weed biomass by 89% compared to the control. Weed biomass response to fertilizer treatment was like the cover crops, with a significant increase in biomass from the N to the N+S treatments. The N fertilizer treatment increased weed biomass by 17% compared to the check plots, and the N+S treatment increased weed biomass by 31% compared to the N fertilizer treatment (Table 3.4). The weed density ANOVA revealed a significant two-way interaction of cover crop and fertilizer treatments. The check plots had the highest weed densities, followed by 'Rymin' rye, winter wheat, triticale, and 'Elbon' rye (Table 3.4). Fertilizer treatment decreased average weed density from the zero-fertilizer treatment to the N fertilizer treatment. However, from the N to the N+S fertilized plots there was a significant increase of nearly 35% in weed density on average. Kumar and Jha (2016) found that increasing nitrogen fertilization rates from 56 up to 168 kg ha⁻¹ increased weed densities in and around barley (*Hordeum vulgare* L.) crops.

The cereal cover crops reduced weed biomass anywhere from 89% up to 98% compared to the fallow control, with ‘Elbon’ rye reducing weed biomass the most. Hayden et al. (2012) found similar results in their study testing cereal rye and vetch (*Vicia* spp.) mixtures, with cereal rye reducing weed biomass by anywhere from 95% to 98%. Werle et al. (2017) revealed that cereal rye can reduce henbit and horseweed biomass by 91% and shepherd’s purse (*Capsella bursa-pastoris* (L.) Medik.) and pinnate tansymustard (*Descurainia pinnata* (Walt.) Britt.) biomass by 95% compared to a fallow control. Cornelius and Bradley (2017) found a 53% and 50% reduction in weed biomass with cereal rye and winter wheat cover crops, respectively. Studies investigating cover crop mixtures have concluded that cover crop weed suppression is often correlated to cover crop biomass. MacLaren et al. (2019) found that overall weed biomass was lower in cover crop mixtures (*Brassica* spp. and *Pisum* spp.) containing at least one cereal (*Secale* spp. and *Avena* spp.). However, the authors concluded that the fast emergence and biomass accumulation of cereals was the primary mechanism of weed suppression, rather than final cover crop biomass at the time of termination. Cover crops have also been shown to reduce weed densities with horseweed density reduced anywhere from 52% to 86% with the use cereal rye and forage radish (*Raphanus sativus* L.) (Wallace et al. 2019). A study conducted by Akemo et al. (2000) using cereal rye and field pea (*Pisum sativum* L.) mixtures and monocultures reduced weed densities by 65% compared to the fallow control over the course of three years. In addition, the pure cereal rye monoculture reduced grass weed densities the most compared to the rye and pea mixture and the pea monoculture.

3.3. Fertilizer Effects on Cover Crop Allelopathy

An analysis of the Palmer amaranth seed germination bioassay results from 2021 revealed that the interaction of cover crop treatment, fertilizer treatment, and extract concentration was not significant. Further analysis of the fixed effects showed that extract concentration was a significant predictor of germination ($P < 0.001$), but not cover crop treatment ($P = 0.33$) nor fertilizer treatment ($P = 0.24$). Overall, the average germination of the Palmer amaranth seeds was 51% in the water control treatment, 35% at the 1% concentration, 22% at the 3% concentration, and 18% at the 5% concentration (Table 3.5). The 5% extract concentration resulted in the greatest germination reduction of Palmer amaranth, 33% relative to the water control (Figure 3.5). The 2022 seed germination bioassay yielded similar results to 2021, with a few exceptions. The ANOVA three-way interaction (cover crop by extract concentration by fertilizer) and all two-way interactions were not significant. Main effects of fertilizer and extract concentration were significant. Increasing extract concentration reduced Palmer amaranth germination dramatically, with the 5% extract reducing germination by 61% compared to the water control (Table 3.5). Nitrogen treatment reduced seed germination the most compared to the no fertilizer and the N+S treatments. Palmer amaranth germination was 26.5% with the nitrogen treatment, 32.1% with the nitrogen and sulfur treatment, and 32.6% with the no fertilizer treatment (Table 3.5).

The hypothesis was that the N and the N+S fertilizer treatments would decrease the allelopathic potential of the cover crop residues, thus increasing the germination of Palmer amaranth seeds. The results do not confirm the hypothesis, as the N fertilizer treatment seemingly increased the allelopathic potency of the cover crops, decreasing Palmer amaranth germination. The N+S treatment did decrease the allelopathic potential of the cover crops as expected, resulting in an increase in Palmer amaranth germination, but it was not significantly

higher than the water control. There was conflicting evidence as to the effect of nitrogen fertilization on cover crop allelopathy. For example, Mwaja et al. (1995) found that the extracts from the rye tissue of plants grown in low fertility were more toxic than that of the tissues from rye plants grown in high fertility in terms of DIBOA per mL. The authors concluded that high nitrogen fertility produces more biomass, but allelochemical content may not necessarily increase. Reburg-Horton et al. (2005) also found higher concentrations of BX's in the no nitrogen treatment compared to other nitrogen rates (22, 45, 90 kg ha⁻¹). On the other hand, Tabaglio et al. (2013) found that the total concentration of BX's applied to the soil through rye residues were 57% and 105% higher in the 250 and 300 kg N ha⁻¹ treatments previously applied for corn. In addition, Gavazzi et al. (1999) also revealed that the BX content in rye residue increased by 41% with a N treatment of 50 kg ha⁻¹. The results of this study fall somewhere in between, with a decrease in germination from the no fertilizer (32.6%) and N+S (32.1%) treatments to the N treatment (26.5%) (Table 3.5). One proposed reason is in the fact that some of the biomass was less in the winter wheat and "Rymin" rye N cover crop treatments than in either of the other fertilizer treatments (Table 3.4). It is possible that the reduction in biomass in these treatments resulting in higher levels of allelochemical concentrations per gram of plant tissue, resulting in more germination than the other two treatments. Further research into the exact quantities of allelochemicals would need to be conducted to confirm this hypothesis.

Increasing extract concentration from 1% to 5% decreased Palmer amaranth germination significantly both years. Studies have shown that higher levels of cover crop residue often result in greater weed suppression, hence the decrease in germination from the 1% extract concentration to the 5% extract concentration (Burgos and Talbert 2000, Petcu et al. 2017, Tabaglio et al. 2013). For example, Petcu et al. (2017) found that increasing extract

concentration from 1.25% up to 10% decreased germination significantly. The authors concluded that the 5% extract concentration was more inhibitory than any of the differences between the wheat and triticale cultivars tested. In general, high fertility regimes may result in greater biomass amounts, but the allelochemical concentrations within the cover crop tissues may not be increased.

Conclusions

The objectives of this study were to document the emergence patterns and timing of winter annual weeds and to evaluate the effects of N+S fertilizers on the allelopathic potential of four different winter cereal cover crops in a no-tillage soybean cropping system. Henbit emergence timing was consistent across years, with most of the seedlings appearing between September 22 and January 1. Mouseear chickweed emerged mostly in the fall, and emerged rapidly, with the highest rate of exponential growth compared to the other weed species. All cover crops reducing the weed biomass anywhere from 89% up to 98% compared to the fallow control. Across all cover crop treatments, “Elbon” rye had the lowest average weed counts observed, the highest average biomass, and the lowest weed biomass. These results suggest that the level of weed suppression by cover crops is correlated to cover crop biomass, which is corroborated by other studies on cover crop monocultures and mixtures (Baraibar et al. 2017, MacLaren et al. 2019, Werle et al 2017). Fertilizer treatment did not influence cover crop biomass in the 2020 to 2021 experimental year, likely due to a late application in the spring and small subplot size. However, fertilizer had a significant influence on cover crop and weed biomass in 2022, with incremental increases from the no fertilizer treatment to the nitrogen and the nitrogen and sulfur treatments. These results are also consistent with others in terms of biomass response to increasing nitrogen fertility (Carciochi et al. 2017, Tabaglio et al. 2013). Although weed population densities and biomass also increased with nitrogen and sulfur fertilizer treatments, the cover crop biomass increase can compensate with increased biomass of its own, thus resulting in adequate weed control (Kumar and Jha, 2016).

The laboratory bioassay experiments revealed that increased extract concentration resulted in decreased Palmer amaranth germination, with reductions occurring incrementally

from the water control to the five percent extract concentration. This suggests that higher amounts of cover crop residue results in more allelochemical leachates released into the soil, potentially resulting in greater weed suppression. These results are supported by multiple bioassay studies, for example, Reuda-Ayala et al. (2015) found that there was stronger inhibition of seed germination with more concentrated extracts. In addition, Burgos and Talbert (2000) found that small-seeded weeds, such as Palmer amaranth, were inhibited more by increasing extract concentration than large-seeded weeds. Fertilizer had variable effects on seed germination, as the results show a decrease from the no fertilizer treatment to the N fertilizer treatment. Studies have shown that low nitrogen increases the phytotoxicity of rye residues (Mwaja et al. 1995, Reburg-Horton et al. 2005), and that high nitrogen enhances the phytotoxicity of rye residues (Gavazzi et al. 2010, Tabaglio et al. 2013). The increase in biomass as a response to the N fertilizer may have diluted the effect of increased BX production in the rye tissues, further research would need to be conducted to quantify the relative amounts of allelochemicals within the rye residues. Overall, results indicate that increasing fertilizer inputs may increase cover crop weed suppression through more cover crop biomass but may not increase the allelopathic potential of the cover crop tissues.

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Figures and Tables

Table 3.1. Monthly rainfall totals (mm) and average monthly temperatures (°C) for the duration of the study and 30-year averages for the USDA PMC near Manhattan, KS.

Month	Rainfall ^{ab}			Temperature ^{ab}		
	2020 - 2021	2021 - 2022	30-yr average	2020 – 2021	2021 - 2022	30-yr average
	mm			C		
August	46	72	86	24.6	26.4	25.0
September	55	69	76	19.3	23.1	20.0
October	17	83	56	11.4	15.5	13.3
November	60	29	36	8.6	8.6	6.1
December	24	8	18	2.2	5.5	-0.6
January	23	7	8	1.3	-0.8	-1.7
February	3	2	18	-3.0	0.1	0.6
March	92	57	43	9.4	6.6	6.7
April	52	26	74	12.1	12.4	12.8
May	134	231	109	16.7	19.0	18.3

^a Weather data obtained from Kansas Mesonet weather monitoring station in Ashland

Bottoms, KS

^b 30-year average weather data obtained from US Climate Data website:
(<https://www.usclimatedata.com/climate/parsons/kansas/united-states/usks0459>)

Table 3.2. Dates of field operations and experiment procedures at the USDA PMC near Manhattan, KS.

Field Operation	Date of Operation		
	2020	2021	2022
Cover Crop Seeding	Sep 24	Sep 28	-
P.V.C. Ring Installation	Oct 9	Oct 19	-
Soil Sampling	Nov 14	Nov 18	-
Fertilizer Application	-	Apr 5	Mar 4
P.V.C. Ring Removal	-	May 12	May 16
Biomass Sampling	-	May 12	May 16/17
Cover Crop Termination	-	May 12	May 23

Table 3.3. Common names, Bayer code, % composition of total, and Relative Abundance (%) of the winter annual weeds observed in the weed rings and the 0.25 m⁻² quadrats throughout the 2020-2021 and 2021-2022 experimental years.

% Of total is the total number of plants for a specific species, divided by the total of all species. Relative Abundance values were averaged over cover crop treatments.^{ab}

Year	Common Name	Bayer Code	% of Total		RA (%)	
			Weed rings	0.25-m ² quadrat	Weed rings	0.25-m ² quadrat
2020-2021	Henbit	LAMAM	92.03	-	73.33 a	-
	Horseweed	ERICA	7.56	-	26.67 b	-
2021-2022	Henbit	LAMAM	83.35	58.52	54.37 a	46.52 a
	Mouseear chickweed	CERVU	8.93	10.71	17.76 b	17.75 b
	Field pansy	VIORA	3.89	9.53	12.62 c	12.87 bc
	Flixweed	DESSO	3.83	3.2	11.00 c	5.82 de
	Carpetweed	MOLVE	1.10	10.85	4.28 d	10.61 cd
	Dandelion	TAROF	-	5.28	-	3.03 de
	Blue mustard	COBTE	-	1.32	-	1.90 de
	Horseweed	ERICA	-	0.56	-	1.49 e

^a Values within a column with different letters indicate significance at p<0.05 (Tukeys HSD)

^b Bayer codes retrieved from WSSA weed database.

Table 3.4. Cover crop and weed biomass (g m⁻²) and weed density (# plants m⁻²) for each cover crop treatment and fertilizer treatment in 2022.

^a Values for each category were averaged over replication.

Cover Crop Treatment	Cover Biomass	Weed Biomass	Weed Densities
	g m ⁻²		# plants m ⁻²
Check	-	95.0 ± 11.1 a	741.3 ± 103.5 a
‘Elbon’ rye	451.7 ± 39.5 a	2.0 ± 0.42 b	142.7 ± 30.3 b
‘Rymin’ rye	321.9 ± 21.5 b	8.7 ± 3.2 b	420.0 ± 120.1 a
Triticale	258.1 ± 21.7 b	5.9 ± 1.4 b	268 ± 39.4 b
Winter wheat	239.7 ± 19.1 b	6.1 ± 0.9 b	344 ± 67.4 b
Fertilizer Treatment			
None	263.3 ± 27.1 a	18.6 ± 4.0 a	354.4 ± 62.2 a
Nitrogen	270.0 ± 19.9 a	21.1 ± 2.9 a	315.2 ± 18.6 a
Nitrogen plus Sulfur	420.4 ± 24.2 b	30.9 ± 6.2 b	480 ± 90.6 a

^a Within each group and year, different letters indicate significant differences at a p<0.05 level (Tukeys HSD).

Table 3.5. Germination (%) of Palmer amaranth from the 2021 and 2022 bioassay experiments based on extract concentration (%), fertilizer treatment, and cover crop treatment.

Cell values are the mean germination within group ^a. Standard error calculated by treatment within year; Extract concentration (n=144), Fertilizer treatment (n= 108) from 2022. Extract concentration (n=60) from 2021, and for both years, water (n=15).

Palmer amaranth germination (%)		
Extract Concentration (%)	Year	
	2021	2022
water	51.2 ± 2.6 a	74.9 ± 2.4 a
1	34.7 ± 1.3 b	45.8 ± 1.1 b
3	22.0 ± 1.2 c	31.7 ± 0.9 c
5	18.2 ± 1.4 c	13.6 ± 0.7 d
Fertilizer Treatment ^b		
None	25.3 ± 1.94	32.5 ± 1.7 a
Nitrogen	25.5 ± 1.26	26.5 ± 1.7 b
Nitrogen and Sulfur	23.9 ± 1.56	32.1 ± 1.5 a
Cover Crop Treatment ^b		
“Elbon” rye	24.5 ± 1.42	31.7 ± 1.69
“Rymin” rye	27.1 ± 2.66	30.5 ± 1.62
“Everest” winter wheat	23.7 ± 2.17	28.9 ± 1.63
“Surge” triticale	26.1 ± 1.86	30.2 ± 1.72

^a Within each group and year, different letters indicate significant differences at a p<0.05 level (Tukeys HSD).

^b Groups without letters of separation were not significantly different.

Figure 3.1. Emergence curves for henbit (LAMAM) and horseweed (ERICA) using cumulative thermal time (TT) for the 2020-2021 experimental year.

Thermal Time (TT) accumulation begins on August 1, base germination threshold temperature (Tbase) for each species listed. R-squared value represents how well the logistic model fits the observed data points.

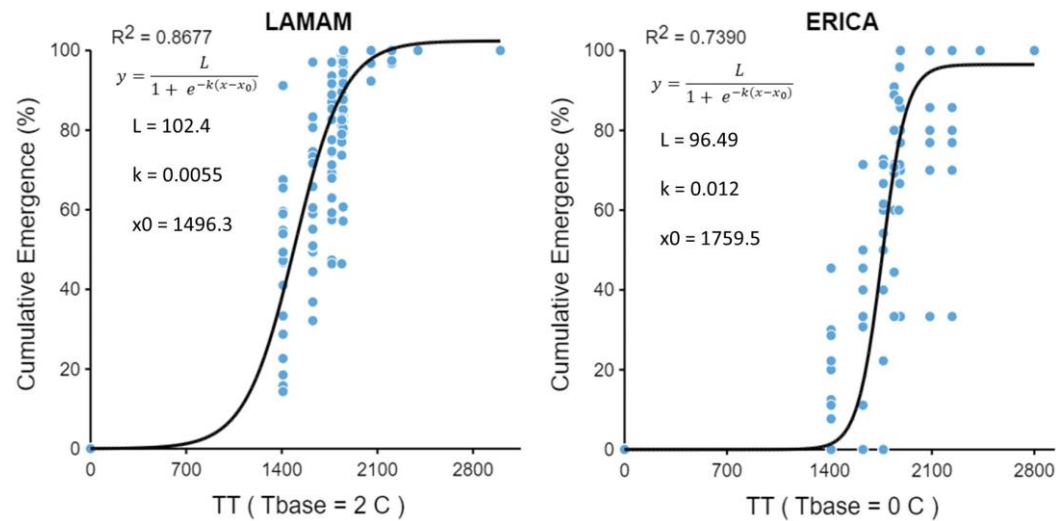


Figure 3.2. Emergence curves for henbit (LAMAM) and mouseear chickweed (CERVU) using cumulative thermal time (TT) for the 2021-2022 experimental year.

Thermal Time (TT) accumulation begins on August 1, base germination threshold temperature (Tbase) for each species listed. R-squared value represents how well the logistic model fits the observed data points.

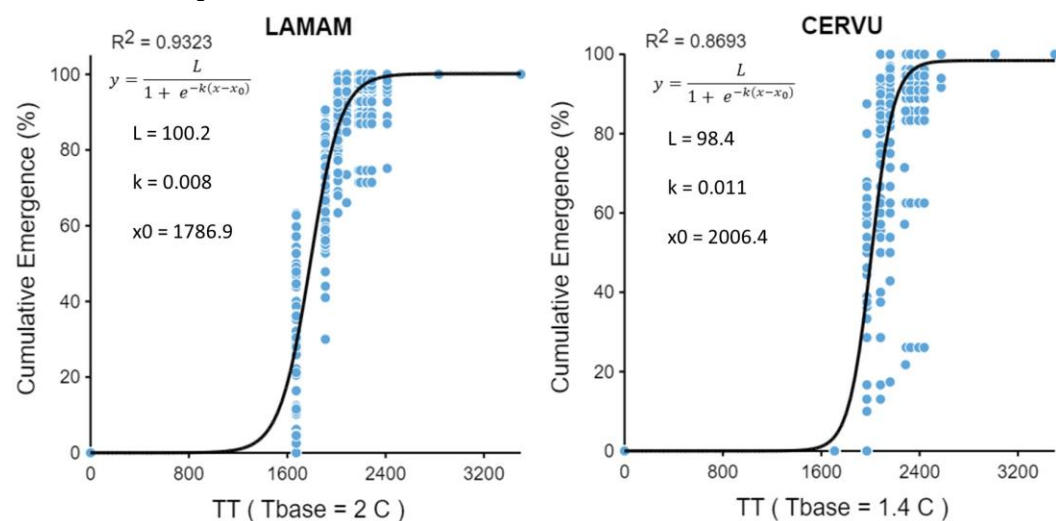


Figure 3.3. Mean total emergence (%) for each of the weed species from the 2020 to 2021 and 2021 to 2022 experimental year at Manhattan, KS.

LAMAM cumulative emergence averaged over the two experimental years. Dashed line separates the strict winter annual species to the left of the line, from the facultative winter annuals to the right of the line. Percent total emergence cutoff from strict versus facultative winter annuals used is 70% in the fall.^a

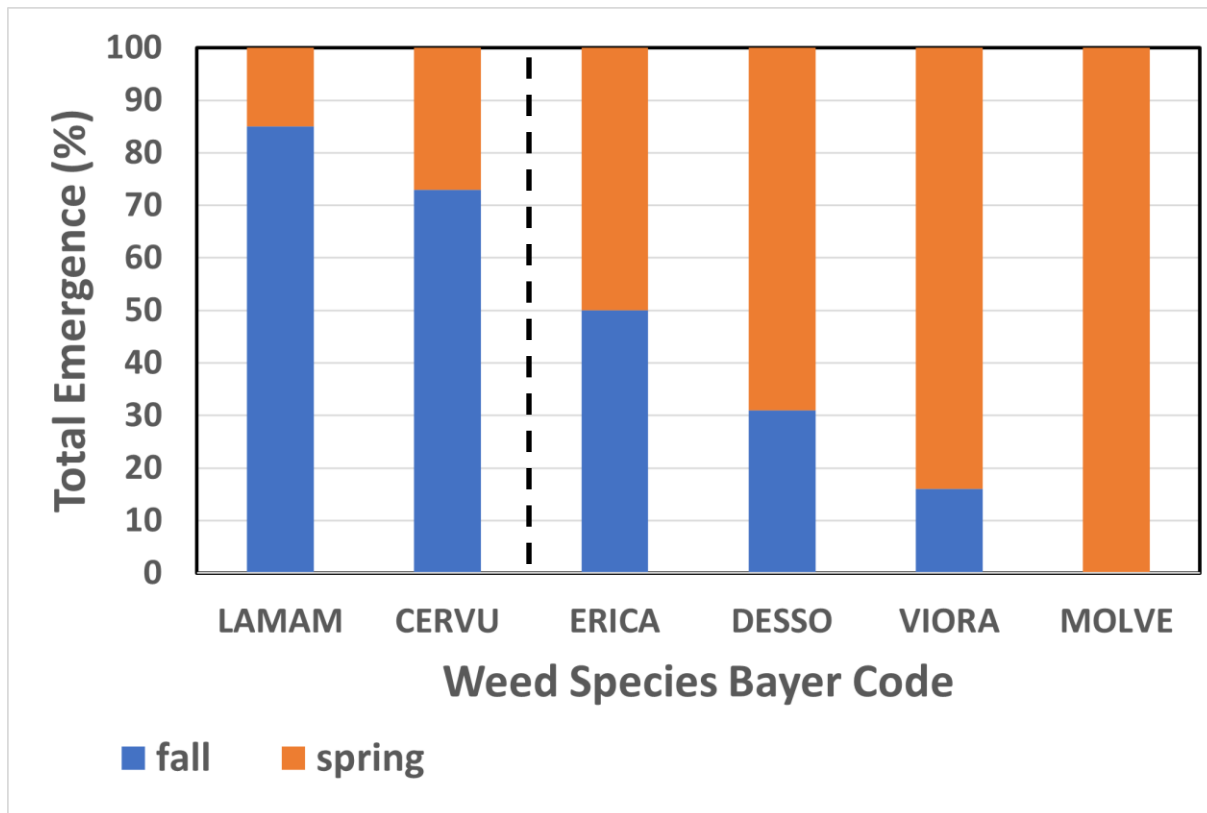
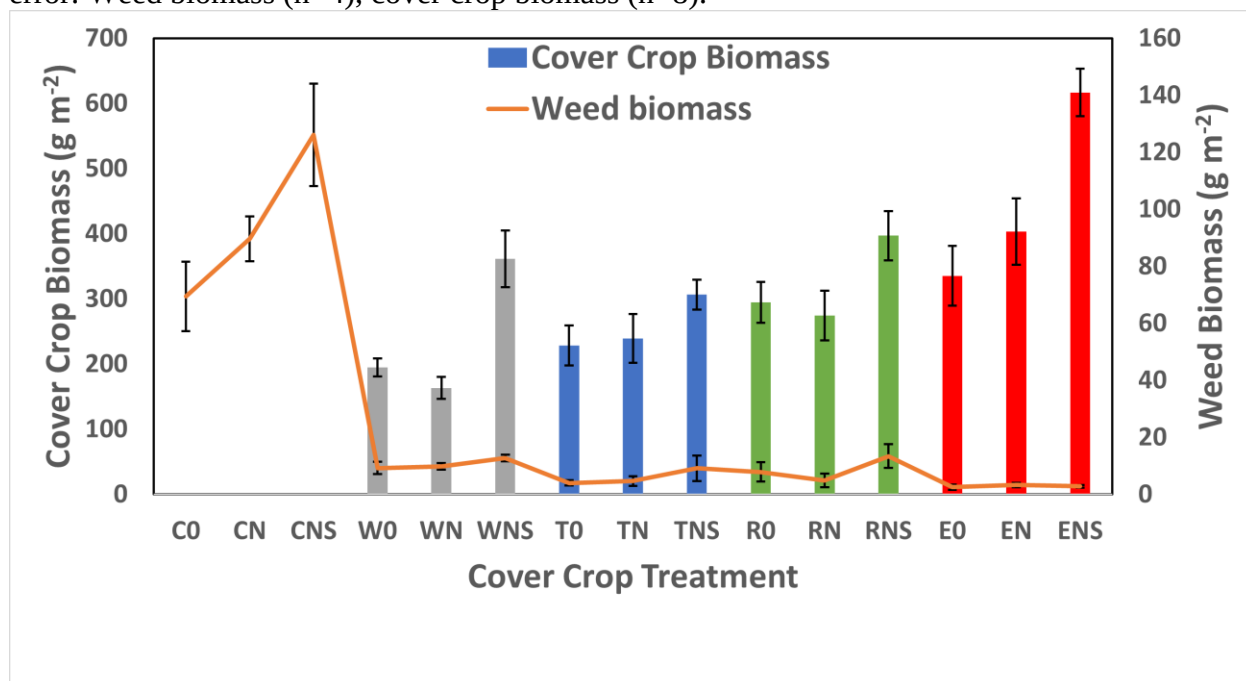


Figure 3.4. Mean cover crop dry weight (g m^{-2}) and mean weed dry weight (g m^{-2}) for the 2022 harvest.

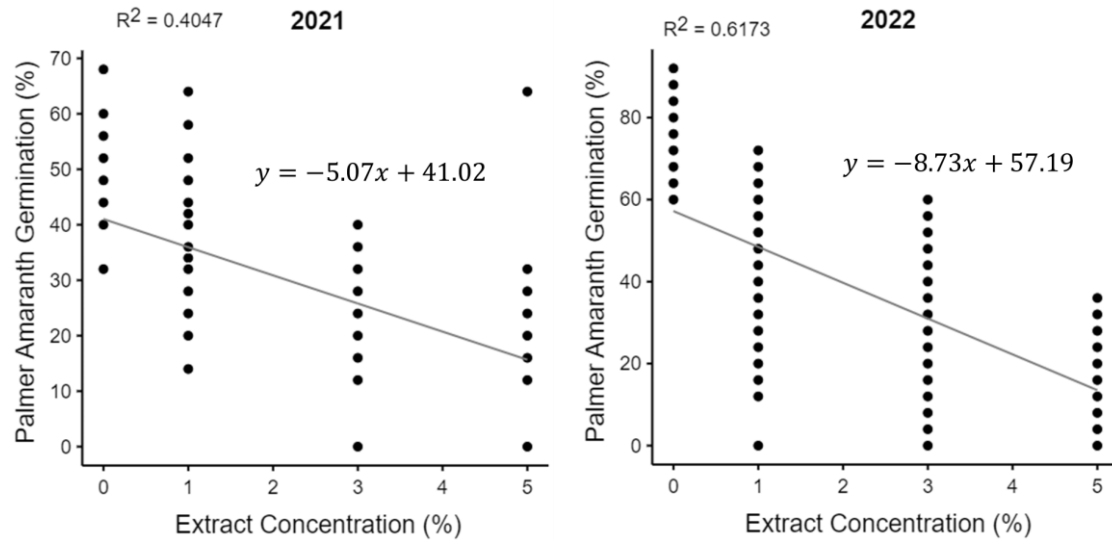
Dry weights averaged over replication for each cover crop treatment. Bars represent standard error: Weed biomass ($n=4$), cover crop biomass ($n=8$). ^a



^a Abbreviations: C0: Check, no fertilizer; CN: Check, Nitrogen; CNS: Check, Nitrogen+Sulfur; E0: 'Elbon' rye, no fertilizer; EN: 'Elbon' rye, Nitrogen; ENS: 'Elbon' rye Nitrogen+Sulfur; R0: 'Rymin' rye, no fertilizer; RN: 'Rymin' rye, Nitrogen; RNS: 'Rymin' rye, Nitrogen+Sulfur; T0: 'Surge' triticale, no fertilizer; TN: 'Surge' triticale, Nitrogen; TNS: 'Surge' triticale, Nitrogen+Sulfur; W0: 'Everest' wheat, no fertilizer; WN: 'Everest' wheat, Nitrogen; WNS: 'Everest' wheat, Nitrogen+Sulfur.

Figure 3.5. Palmer amaranth germination (%) by extract concentration for the 2021 and 2022 seed germination bioassay experiments.

Zero percent extract concentration represents the water control. Line of best fit modelled with simple linear regression with r-squared to show strength of fit to the model.



Appendix A - Chapter two ANOVA Tables

Biomass Results:

Table A.1. Cover Crop Biomass for Parsons, KS, 2021.

1-way ANOVA results:			
Source of Variation	df	F-score	P-value
Between Groups	49	31.8558	< 0.00000001
Residual	30		

Table A.2. Weed Density for Parsons, KS, 2021.

1-way ANOVA results:			
Source of Variation	df	F-score	P-value
Between Groups	9	4.8679	0.0005
Residual	30		

Table A.3. Cover Crop Biomass for Parsons, KS, 2022.

1-way ANOVA results:			
Source of Variation	df	F-score	P-value
Between Groups	9	13.5123	0.00000002
Residual	30		

Table A.4. Weed Biomass for Parsons, KS, 2022.

1-way ANOVA results:			
Source of Variation	df	F-score	P-value
Between Groups	9	10.3481	0.00000047
Residual	30		

Table A.5. Weed Density for Parsons, KS, 2022.

1-way ANOVA results:			
Source of Variation	df	F-score	P-value
Between Groups	9	1.9723	0.079
Residual	30		

Table A.6. Cover Crop Biomass for Parsons, KS, 2021-2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	9	37.6904	< 0.00000001
Year	1	345.8021	< 0.00000001
Cover*Year	9	23.3283	< 0.00000001
Residual	60		

Table A.7. Weed Densities for Parsons, KS, 2021-2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	9	1.8497	0.0776
Year	1	55.7724	< 0.00000001
Cover*Year	9	2.2063	0.0339
Residual	60		

Weed Counts and RA:

Table A.8. Weed Counts for Parsons, KS, 2021.

Type 3 Tests of Fixed Effects				
Effect	Num df	Den df	F-value	P-value
Cover Crop	9	27	2.93	0.4064

Table A.9. Weed Counts for Parsons, KS, 2022.

Type 3 Tests of Fixed Effects				
Effect	Num df	Den df	F-value	P-value
Cover Crop	9	27	4.84	0.051

Table A.10. Weed Count RA for Parsons, KS, 2021.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	9	0.13	0.9988
Weed	6	13.10	<0.0001
Cover*Weed	54	1.34	0.0772

Table A.11. Weed Count RA for Parsons, KS, 2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	9	0.29	0.9779
Weed	6	32.77	<0.0001
Cover*Weed	54	1.95	0.0005

Table A.12. Weed Counts for Parsons, KS, 2021-2022.

Type 3 Tests of Fixed Effects				
Effect	Num df	Den df	F-value	P-value
Cover Crop	9	66	4.42	0.0002

Table A.13. Weed Count RA for Parsons, KS, 2021-2022.

Type 3 Tests of Fixed Effects				
Effect	Num df	Den df	F-value	P-value
Cover Crop	9	419	0.29	0.9776
Weed	5	419	28.36	<0.0001
Cover *Weed	45	419	1.63	0.0082

Appendix B - Chapter Three ANOVA tables

Biomass Results:

Table B.1. Cover Crop Biomass for Manhattan, KS, 2021.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	4	0.2788	0.8902
Fertilizer	2	1.8767	0.1649
Cover*Fertilizer	8	1.0063	0.4448
Residual	45		

Table B.2. Cover Crop Biomass for Manhattan, KS, 2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	4	80.2835	< 0.00000001
Fertilizer	2	24.9147	< 0.00000001
Cover*Fertilizer	8	3.391	0.0017
Residual	105		

Table B.3. Weed Biomass for Manhattan, KS, 2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	4	67.8887	< 0.00000001
Fertilizer	2	3.6782	0.0331
Cover*Fertilizer	8	1.9635	0.0735
Residual	45		

Table B.4. Weed Densities for Manhattan, KS, 2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	4	12.2303	0.00000082
Fertilizer	2	2.9876	0.0605
Cover*Fertilizer	8	2.6141	0.0193
Residual	45		

Weed Counts and RA:

Table B.5. Weed Counts for Manhattan, KS, 2021.

Type 3 Tests of Fixed Effects				
Effect	Num df	Den df	F-value	P-value
Cover Crop	4	27	3.51	0.067
Weed	1	27	28.36	<0.0001
Cover *Weed	4	27	1.63	0.0888

Table B.6. Weed Counts for Manhattan, KS, 2022.

Type 3 Tests of Fixed Effects			
Effect	Num df	Den df	P-value
Cover Crop	4	222	0.0578
Fertilizer	2	222	0.3182
Cover*Fertilizer	8	222	0.3836
Weed	4	222	<0.0001
Cover*Weed	16	222	0.1060
Fertilizer*Weed	8	222	0.3051
Cover*Fertilizer*Weed	32	222	0.8323

Table B.7. Weed Count RA for Manhattan, KS, 2021.

Type 3 Tests of Fixed Effects				
Effect	Num df	Den df	F-value	P-value
Cover Crop	4	27	0.00	1.000
Weed	1	27	224.51	<0.0001
Cover *Weed	4	27	1.37	0.2719

Table B.7. Weed Count RA for Manhattan, KS, 2022.

2-way ANOVA results:			
Source of Variation	df	F Score	P-value
Cover Crop	14	2.90	0.0005
Weed	4	29.54	<0.0001
Cover*Weed	56	3.16	<0.0001

Bioassay Results:**Table B.8. 2021 bioassay.**

Type 3 Tests of Fixed Effects			
Effect	Num df	Den df	P-value
Extract	2	108	<0.0001
Cover	3	108	0.5240
Extract*Cover	6	108	0.7257
Fertilizer	2	108	0.7722
Extract*Fertilizer	4	108	0.5388
Cover*Fertilizer	6	108	0.1106
Extract*Cover*Fertilizer	12	108	0.6028

Table B.9. 2022 bioassay.

Type 3 Tests of Fixed Effects			
Effect	Num df	Den df	P-value
Extract	2	394	<0.0001
Cover	3	394	0.3017
Extract*Cover	6	394	0.2866
Fertilizer	2	394	<0.0001
Extract*Fertilizer	4	394	0.1026
Cover*Fertilizer	6	394	0.4377
Extract*Cover*Fertilizer	12	394	0.2321