INTEGRATED WEED MANAGEMENT FOR THE SUPPRESSION OF RHIZOMATOUS PERENNIAL WEEDS IN ORGANIC AGRICULTURE

by

Kara Ann Hettinger

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ABSTRACT

Convolvulus arvensis (L.) (field bindweed) and *Cirsium arvense* (L.) Scop. (Canada, creeping, or Californian thistle) are the most problematic weeds for organic grain farmers in semi-arid Montana and the wider Northern Great Plains (NGP) due to their rhizomatous, perennial root systems. Historically, intensive tillage was used to disrupt and control the root systems. An integrated approach, which combines biological, cultural, and mechanical controls, has been recommended to manage difficult perennial weeds while reducing reliance on a singular management strategy like intensive soil cultivation. Two associated studies were conducted to assess *C. arvensis* and *C. arvense* responses to different combinations of tillage, grazing, and crop sequences. In both studies, treatments were arranged on a spectrum of crop competition and tillage intensity, which were inversely related. For example, a two-year sequence of perennial alfalfa (*Medicago sativa* L.) constituted maximum crop competition and minimal tillage intensity, whereas wheat (*Triticum* spp.) followed by two consecutive years of tilled fallow represented minimal crop competition and maximum tillage intensity. Treatments within this gradient had crop sequences with varied mixtures of annual and biennial monocot and dicot crops and minimal to moderate tillage regimes, sometimes including livestock grazing with sheep (*Ovie aries*).

Tilled fallow and multi-year alfalfa treatments prevented or decreased both *C. arvensis* and *C. arvense* populations over three-year periods across the two studies. For *C. arvensis*, a treatment using a biennial sweet clover crop and livestock grazing for crop and weed termination increased *C. arvensis* density. Crop sequences with multiple years of annual monocot or dicot crops increased *C. arvense* density and biomass over time. There were no differences in *C. arvense* populations due to tillage method (e.g., standard or reduced). Both studies demonstrated that intensive soil cultivation or intensive crop competition in the form of perennial alfalfa were most successful in suppressing perennial weeds. Highly diverse annual or biennial crop rotations, under either standard or reduced tillage methods, did not prevent population perennial *C. arvensis* or *C. arvense* from increasing. Incorporating a perennial forage or temporary pasture phase into annual organic grain rotations of the NGP is recommended to reduce perennial weed pressure.

CHAPTER ONE

INTRODUCTION TO THESIS

Since the passing of the Organic Food Production Act (OPFA) in 1990, the enterprise of organic agriculture has continued to experience worldwide growth (Reganold and Wachter 2016). In two decades, from 1999 to 2019, organic agricultural land worldwide increased from 11 million hectares to over 72 million hectares (Willer et al. 2021). The United States had over 2.3 million hectares of organically managed farmland in 2019. In the same year, organic food sales in the United States topped 50 billion dollars – the largest organic market in the world (Willer et al. 2021) – with consumer demand growing year-to-year (Hughner et al. 2007; NASS 2019).

Weeds are often considered one of the biggest threats to crop production in organic systems (Bàrberi 2002; Gomiero et al. 2011). Organic crop yield, on average, is 20% less than that of conventional yield and is often attributable to weed pressure and soil fertility issues (de Ponti et al. 2012; Seufert et al. 2012). Additionally, organic agricultural operations often have a greater abundance and diversity of weedy species than conventional operations (Bengtsson et al. 2005; Larson et al. 2021; Ngouajio and McGiffen 2002; Pollnac et al. 2008). Developing successful weed management strategies will be crucial for the continued growth of sustainable organic farming.

Agriculture in the Semiarid Northern Great Plains

In 2021, Montana had over four million hectares of operational annual cropland (NASS 2022). Less than 3% of farmland in Montana (129,000 hectares) is in certified-organic crop production (NASS 2021), but still ranks in the top three states with the most certified-organic

acreage (NASS 2021). In Montana and the broader Northern Great Plains (NGP) region, where precipitation is the limiting factor for crop selection and growth, drought-resistant spring wheat is the primary cash crop (NASS 2018; Padbury et al. 2002). For most major field crops, like corn and soybean, the added economic costs of operating in organic production are overcompensated by returns (McBride et al. 2015). However, the economic costs of growing organic grain are not always offset by higher returns (McBride et al. 2015). In a study from the mid-Atlantic region of the United States where precipitation is not a limiting condition, organic wheat still infrequently yielded higher than conventional wheat, highlighting the need for premium pricing (Cox et al. 2019). In a study from the Northern Great Plains, the highest price premiums were required for the most profitable organic phase to outcompete the most profitable conventional rotation (Smith et al. 2004).

Dryland crop production in the NGP can be particularly challenging due to difficult growing conditions. The climate is characterized by long, cold winters, short and hot summers, persistent strong winds, and low and erratic precipitation (Padbury et al. 2002; Tanaka et al. 2010). Most rainfall events take place from April to September, with relative humidity remaining low throughout the growing season (Peterson and Westfall 2005; Tanaka et al. 2010). Historically, small grain growers in the NGP managed low precipitation conditions using a tilled fallow period as a risk management practice to retain adequate soil moisture for the following year's wheat crop (Tanaka et al. 2010). Wheat-fallow acreage peaked at 16 million hectares in 1971, with an ensuing, steady decline in most areas of the NGP due to the proliferation of chemical herbicides and fertilizers, market expansion, and soil resource concerns (Tanaka et al. 2010). With the advent of glyphosate and other herbicides, conventional producers were able to

maintain a no-till chemical fallow for weed control and soil water retention (Donald and Prato 1991; Hansen et al. 2012).

For organic producers with no systemic herbicide option, tillage has remained a key component of weed management strategies though erosion is a major concern. Consistent tillage (e.g., five or more tillage events in a season) has shown success in managing weed populations during fallow periods. Early studies on the efficacy of intensive tillage demonstrated near-eradication of perennial weeds but required upwards of five tillage events annually (Hodgson 1958; Wiese and Rea 1959). As the interest in soil quality has increased in agricultural science, concerns about the negative effects of tillage on soil have also emerged (Lehmann et al. 2020; Sherwood and Uphoff 2000). Conventional tillage often utilizes chisel plows and tandem disks to invert soil. Typically, minimal tillage systems either do not invert soil or do so minimally to retain crop residues on the soil surface (Carr et al. 2013; Hofmeijer et al. 2019). Under semi-arid conditions, conventionally tilled fields have substantially less aggregate stability, soil organic carbon, microbial biomass, and slower infiltration rates, all of which leads to decreased water storage capability and less fertile soil (Liebig et al. 2004; Mitchell et al. 2017). In wind-prone areas like the NGP, minimal or no-till fields also show less soil erosion than their conventionally tilled counterparts (Moyer et al. 1994; Seitz et al. 2018). Weed increases due to reductions in tillage can be significant (Berner et al. 2008; Moyer et al. 1994). In the NGP, perennial rhizomatous weeds like *Convolvulus arvensis* L. Scop. (field bindweed) and *Cirsium arvense* L. Scop. (Canada, creeping, or Californian thistle) have become major production issues in organic systems (Davis et al. 2018; OAEC 2013; Orloff et al. 2018; Tautges et al. 2017).

Perennial Weeds

The perennial weeds *C. arvensis* and *C. arvense* have been detrimental to organic producers worldwide, but especially those in the NGP region (Kniss et al. 2016; OAEC 2013; Tautges et al. 2016; Verschwele and Häusler 2004). The ability of both species to access resources deep in the soil profile, regrow from disrupted root fragments, and produce many viable seeds has led to their success in competing with shallower-rooted annual crops in semi-arid, small-grain organic systems. Organic producers in Montana indicated perennial weed management was a serious challenge, and subsequent reviews of *C. arvensis* and *C. arvense* were conducted (Davis et al. 2018; Orloff et al. 2018). The reviews suggested that incorporating multiple weed management approaches into organic systems may be more successful in suppressing these two perennial rhizomatous weeds compared to a single management strategy, but integrated options were far less prevalent in the scientific literature. Further research analyzing and comparing integrated weed management strategies is therefore imperative.

In agricultural systems, weed community composition is influenced by practices like tillage regimes or crop choice (Blackshaw et al. 1994; Larson et al. 2021; Menalled et al. 2001; Ulber et al. 2009). In conventional no-tillage systems, some perennial weeds of concern can be managed with chemical, post-emergent herbicides (Bayat and Zargar 2020; Donald 1990; Westra et al. 1992). In reduced or no-tillage systems that operate without the use of herbicides, there have been mixed results on the management of perennial weeds. In a meta-analysis performed by Thomas et al. (2004), *C. arvense* and perennial sowthistle (*Sonchus arvensis* L.) were found to be positively associated with reduced and no-till systems on the Canadian Prairies. Carr et al. (2012) found similar increases in both established and new perennial weed populations in

organic no-till systems in the NGP. Often, organic reduced or no-tillage systems either act to spread live rhizome through infrequent mechanical cultivation, or leave the extensive perennial root system intact, both of which allow for the proliferation of perennial weeds across agricultural landscapes.

Perennial weeds can still be problematic in other tillage systems. In a maize-soybean rotation, Tuesca et al. (2001) demonstrated that perennial weeds showed inconsistent behavior in response to both no-tillage and standard tillage systems. A study performed in the same year in wheat systems of the Canadian Prairie also found different perennial weeds to be positively associated with different tillage systems (Blackshaw et al. 2001). In this study, rhizomatous *C. arvense* was dominant in conventional tillage systems, while tap-rooted perennial dandelion (*Taraxacum officinale* L.) and *Sonchus arvensis* were associated with zero-tillage systems. It has been proposed that perennial weeds which resprout from live root fragments may still be problematic in conventional tillage systems because the current intensity and frequency of mechanical cultivation is insufficient for reduction of perennial root systems (Moyer at al. 1994).

Convolvulus arvensis

History, Distribution, and Habitat

Convolvulus arvensis is a Eurasian perennial vine. The species was introduced to the United States in the early to mid-18th century, likely as a seed contaminant (as reviewed by Sosnoskie et al. 2020). By the early 19th century, *C. arvensis* was commonly found throughout the Eastern seaboard and as far as California by 1850 (Sosnoskie et al. 2020). *Convolvulus arvensis* was first collected in Missoula County in 1891 and has since been reported in every county in

Montana (Davis and Orloff 2019). *Convolvulus arvensis* is now a common nuisance species in temperate growing zones worldwide (Sosnoskie et al. 2020).

Convolvulus arvensis is known to inhabit a wide range of ecosystems and climates, including tropical, Mediterranean, and temperate zones (Holm et al. 1977). The weed is considered most detrimental in cereal and small grain cropping systems in temperate agricultural areas (Weaver and Riley 1982). *Convolvulus arvensis* persists in sunny, open areas with moist and fertile to dry and nutrient-poor soils but cannot sustain exposure to water-logged soils (Weaver and Riley 1982). *Convolvulus arvensis* occurs in most ecosystems disturbed by humans, including cultivated farm fields, pastures, residential lawns, waste areas and rights-of-way, vineyards, and orchards.

Growth and Development

Convolvulus arvensis is a perennial, flowering herbaceous vine in the Convolvulaceae family. The vertical roots of *Convolvulus arvensis* can reach up to 4 m, with radially and horizontally spreading roots taking up the top 30-cm of the soil profile (Frazier 1943). *Convolvulus arvensis* rapidly expands across fields through its extensive, adventitious root system and vining nature. Infrequent cultivation may lead to the further spatial expansion of *C. arvensis* rhizomes across agricultural fields. *Convolvulus arvensis* can be a prolific seeder, with seeds remaining viable in soil up to two decades (as reviewed by Weaver and Riley 1982).

Convolvulus arvensis can exhibit vigorous vegetative regrowth in addition to reproduction through seeds (Omezine and Harzallah-Skhiri 2010). *Convolvulus arvensis* has a high level of genetic variability among populations, which can determine a plant's capability to reproduce through its rhizomes (Gaskin et al. 2023). Degennaro and Weller (1984) found that

across five distinct *C. arvensis* biotypes, vegetative reproduction potential varied from 1.8% to 74.5%. Studies into the genetic diversity and subsequent physiological reproductive responses of *C. arvensis* reiterate the species is well-adapted to rapid spread throughout agroecosystems.

Cirsium arvense

History, Distribution, and Habitat

Cirsium arvense is an introduced perennial forb native to Eurasia. *Cirsium arvense* was likely first brought to Canada by the French as a crop seed contaminant in the early 17th century (as reviewed by Moore 1975). The species is believed to have spread quickly into the United States (Guggisburg et al. 2012; Hodgson 1968) or was brought to the United States independently (Hansen 1918). *Cirsium arvense* was promptly understood to be an agricultural nuisance, and subsequent weed control legislation was established in Vermont by 1795 and in New York by 1831 (Moore 1975). The species is now common throughout North America, Europe, North Africa, central Asia, Australia, and New Zealand in agricultural, rangeland, and wildland ecosystems. *Cirsium arvense* was first reported in Montana in 1881 and is currently found in every county (Jacobs et al. 2007).

Cirsium arvense is widespread in plant communities throughout North America and often grows in mesic environments, and most commonly in recently disturbed, nutrient-rich sites. Many humans disturbed areas, including roadways, railways, forests, prairies, and riparian areas are among the most common habitats (Moore 1975; Tiley 2010). Most frequently, *C. arvense* is considered a serious agricultural weed in temperate growing zones. In these agricultural landscapes, *C. arvense* is found frequently in pastures, rangelands, and especially in cereal and small grain crop fields (Donald 1990; Moore 1975).

Growth and Development

Cirsium arvense is a perennial, flowering herbaceous forb in the Asteraceae family. *Cirsium arvense* spreads vigorously through its horizontal, rhizomatous roots and has a recorded vertical rooting depth of 2 m (Donald 1994). Horizontal roots can develop many adventitious flowering shoots throughout a growing season (Donald 1994). Root fragments of only 10 mm in length and 1 mm in diameter have been shown to produce shoots (Hamdoun 1972). In addition to vegetative spread, *C. arvense* can be a prolific seeder, producing anywhere from 600 to 6,000 viable seeds per stem (Heimann & Cussans 1996).

Cirsium arvense weediness in disturbed areas and agricultural fields is most likely attributed to its vigorous vegetative propagation and highly variable physiology (Moore 1975). *Cirsium arvense* can spread readily through disturbance of its perennial root system, primarily by tillage (Gustavsson 2008; Hamdoun et al. 1970; Thomsen et al. 2010). Research has reaffirmed minimal to moderate mechanical cultivation of soil is responsible for root fragment displacement across agricultural fields (as reviewed by Donald 1994). Initial investigation into genetic variation of *C. arvense* has shown intermediate to high levels of genetic diversity among populations (Hettwer and Gerowitt 2004; Solé et al. 2004). Better understanding of the levels of genetic diversity within and between both *C. arvense* and *C. arvensis* populations may be important in determining the most successful management strategies for these weed species.

Integrated Weed Management in Organic Cropping Systems

To maintain minimal weed pressure on crops, weed management tactics may need to be combined. Integrated weed management (IWM) is defined by the combination of two or more weed management strategies, including biological, cultural, and mechanical controls, to

successfully curb weed populations (Buhler 2002; Harker and O'Donovan 2013; Swanton and Weise 1991). Recent meta-analyses performed for both weed species have shown integrated management strategies to be more successful than reliance on a singular tactic, such as mechanical tillage or crop competition, but is understudied in the literature (Davis et al. 2018; Orloff et al. 2018). Determining the most beneficial combination of management tactics will be crucial for the continued success of organic producers in the NGP.

Mechanical Controls

Soil cultivation with machinery is one of the oldest weed management techniques in the history of human agriculture (Timmons 1970). In the early dryland agricultural systems of the NGP, wheat-fallow systems were the predominant method of farming due to its ability to retain soil moisture in low precipitation conditions. Intensive mechanical cultivation of soil was crucial for weed suppression during fallow periods. Highly disruptive moldboard plows and disk harrows were the most common implements used to suppress weeds through inversion and incorporation of soil (Derksen et al. 2002; Tanaka et al. 2010). To successfully manage weeds in early systems, intensively tilled fallow fields could receive anywhere from five to 15 cultivation operations in a season (e.g., Salmon et al. 1953). Though beneficial for dryland wheat farming for a time, such exhaustive practices led to unprecedented levels of soil erosion in the Dust Bowl (Hargreaves 1993; Tanaka et al. 2010).

Subsequently, starting in the late 1930s and continuing well into the 1980s, the practice of stubble mulching became more prevalent as a method to reduce soil erosion on fallowed fields (Allen and Fenster 1986; Gebhardt et al. 1985; McCalla and Army 1961). Stubble mulching is a form of non-inversion tillage where crop residue is left on the field in the fallow period. Such

conservation tillage systems, while helpful in reducing soil erosion and water loss, struggled to suppress perennial weeds like *C. arvensis* and *C. arvense* (Gebhardt et al. 1985). With the increased use of glyphosate in the early 1990s, chemical no-tillage systems became more popular with conventional farmers in the NGP due to reduced labor costs and excellent weed management provided by the herbicide treatments (Carlyle 1997; Kappler et al. 2005; Tanaka et al. 2010).

Other mechanical controls, like well-timed mowing or cutting, have not yet demonstrated control of *C. arvensis*. A mowing study performed in Colorado's semi-arid grasslands showed perennial *C. arvensis* increasing two-fold in spring-mowed areas and increasing nearly three-fold in plots that were mowed three times in a growing season (Prevéy et al. 2014). It is hypothesized that as a prostrate vine *C. arvensis* can avoid serious defoliation in mowing events (Jacobs 2007; Prevéy et al. 2014). A meta-analysis on *C. arvensis* management showed mowing to be an ineffective control in perennial systems and was not represented as a sole management technique in annual cropping systems (Orloff et al. 2018). The lack of literature evaluating mowing as a method for *C. arvensis* suppression suggests that either the approach has not yet been analyzed sufficiently by researchers or the applicability of integrating mowing into an annual cropping system as a weed control measure for *C. arvensis* is not high.

Unlike *C. arvensis,* mechanical controls like cutting or mowing have a greater potential to manage erect *C. arvense* populations, especially when coupled with other management techniques or performed multiple times in a growing season. In a study conducted on permanent pasturelands in New Zealand, 14 of 21 possible twice-a-year mowing combinations produced significant population declines in *C. arvense* (Bourdôt et al. 2016). Unfortunately, most mowing

studies are based on broad seasonal suggestions (e.g., first thistle bolt) instead of a deeper understanding of *C. arvense* root carbohydrate responses to such mowing events (Graglia et al. 2006). In a recent greenhouse study aiming to bridge the knowledge gap, Verwijst et al. (2018) used leaf-stage to suggest when mowing would be most impactful. They found that mowing just before reaching the three to four leaf-stage would greatly reduce *C. arvense* root and rhizome mass. However, implementing a mowing event at such an early life stage may not be realistic for large-scale organic producers. Other recent developments in weed-cutting machinery specifically for cereal crops (e.g., CombCut) suggest that such tools could be advantageous in managing more erect perennial weeds like *C. arvense* due to improved management of both seed production and aboveground biomass (Tavaziva et al. 2019; Verwijst et al. 2017).

Cultural Controls

Another major component of an integrated weed management plan is the use of cultural controls. A primary objective for successful cultural control is to aid in crop competitive advantage over weedy nuisance species (Pavlović et al. 2022; Swanton and Weise 1991). One such tactic is the use of integrating more competitive crops into diverse crop rotations. Benefits of crop rotations can include increasing soil fertility, organic matter, and pest prevention (Bullock 1992). When used properly, crop rotations, or the practice of growing a sequence of crop types on the same field, can also act as an important weed management strategy (Blackshaw et al. 2005; Bullock 1992; Dury et al. 2012).

Perennial weed populations often impact organic cereal grain farms, which are generally characterized by a high number of cereal crops or broadleaf annual crops like lentil (*Lens culinaris* Medik.) or field pea (*Pisum sativum* L.) (Moreby et al. 1994; Verschwele and Häusler

2004). Research into weed suppression using cover crops in irrigated and high-precipitation organic systems has been mixed. A study found the presence of leguminous mono- or polyculture cover crops to cause a reduction in overall weed density from 19% to 87%, though weed species were not specified (Dorn et al. 2015). Winter annual cover crop residue can provide early-season annual weed suppression but has not been successful at full-season control (Carr et al. 2012; Teasdale 1996). In the subhumid area of the NGP, a six-year, continuous organic cropping system using cover crops as a weed suppressant led to increases in weed biomass and weed species richness over time (Halde et al. 2015). In semi-arid dryland agricultural areas of the NGP, established perennial weed populations have not been suppressed by killed cover crop mulch (Carr et al. 2012).

Certain cover crops, such as quick-growing sudangrass (*Sorghum sudanese*; *Sorghum bicolor*), can cause substantial decreases in perennial weed density (Bicksler and Masiunas 2009; Wedryk and Cardina 2012). In ideal growing conditions, sudangrass rapidly grows to a height of 1 m, which can allow it to shade out both *C. arvensis* and *C. arvense* (Bicksler and Masiunas 2009; Stahler 1948). However, while frequently used in the Central and Southern Great Plains, lack of moisture and a short growing season limit the use of sorghum-sudangrass in the NGP (Carr et al. 2020; Meccage et al. 2019). Biennial yellow sweet clover (*Melilotus officinalis* L.) as a green manure-grown cover crop has lowered weed densities 75% to 97% and was particularly adept at suppressing perennial weeds (Blackshaw et al. 2001) but is not well represented in literature.

For perennial weeds specifically, perennial forage or temporary pasture phases integrated into annual cropping systems have shown successful management (Favrelière et al. 2020;

Ominski et al. 1999; Tautges et al. 2017). In a humid climate, a perennial grass-alfalfa mixture demonstrated significant control of perennial weeds when compared to varying tillage methods (Grosse et al. 2021). In past studies, mown alfalfa (*Medicago sativa* L.) in a semi-arid climate was considered an incredibly effective control for *C. arvense* due to the combined efforts of defoliation and direct competition (Hodgson 1958). Contrarily, however, Barroso et al. (2015) found semi-arid grazed perennial systems to limit the proliferation of annual weeds but increase the presence of perennial weeds. Additionally, special consideration needs to occur for the use of perennial forages as weed suppressants, as they can substantially reduce following grain yields in water-limited environments (Entz et al. 2002).

Biological Controls

A third pillar of integrated weed management in organic systems is biological control. Grazing by livestock, when available and practical, can be a vital biological control method to manage weeds in agroecosystems. While best applied in pasturelands, grazing has been proposed as a suitable weed removal technique for arable cropping fields (Popay and Field 1996). Though livestock grazing with sheep has been recommended as a method of weed control in small grain and cereal production systems, initial research results have been mixed and practical integration in the NGP is currently limited (Krall and Schuman 1996; Larson et al. 2021; Miller et al. 2015). Miller et al. (2015), for example, found that while overall weed pressure was reduced in grazed wheat-fallow systems, perennial weeds became more abundant. Furthermore, livestock integration into a more diversified, annual hay crop-fallow-wheat rotation saw an unacceptable increase in weed pressure and subsequent 51% reduction in grain yield (Miller et al. 2015). Research conducted in the same area of Montana found a grazed, continuous spring wheat

system had the highest weed abundance compared to chemically and mechanically managed systems (Barroso et al. 2015). A third study conducted in the area demonstrated that a grazed, highly diverse organic cropping system produced higher weed biomass, greater weed species richness, and greater weed species diversity (Larson et al. 2021). Further research is required to fully understand both the ability of grazing for weed control in a broader range of crop sequences (e.g., more to less diverse) and the practicality of organic farmers integrating livestock into their cropping systems.

In addition to the use of livestock grazers, biological controls can use herbivorous insects and fungal and bacterial pathogens (Eilenberg et al. 2001). Research into biological management of *C. arvensis* initially produced numerous potential control organisms (Rosenthal 1981). A gall mite (*Aceria malherbae*), with and without a secondary treatment (e.g., sublethal herbicide application, under-seeded cover crop) has demonstrated varied levels of success in suppression of *C. arvensis* and is not yet implemented outside of laboratory studies (Rosenthal and Platts 1990; Boydston and Williams, 2005). The fungal pathogen *Stagonospora convolvuli* has demonstrated efficacy in non-agricultural perennial systems (Boss et al. 2007). In annual agricultural systems, fungal pathogens of the *Phoma* species (*Phomopsis convolvulus*, *Phoma proboscis*) have effectively reduced *Convolvulus arvensis* abundance (Heiny 1994; Vogelsgang et al. 1998). Despite field and laboratory success, no biological control is currently used in agricultural settings for *C. arvensis* suppression.

The history of biological control for *C. arvense* spans 50 years, with the initial release of a defoliating flea beetle (*Altica carduorum*) (as reviewed by Cripps et al. 2011). Though initially promising, subsequent studies of *A. carduorum* populations showed low reproductive rates, high

levels of predation, and high mortality at all life stages, associated with poor adaptation to cooler, northern climates (Baker et al. 1972; Peschken 1977). The successive release of a stem-mining weevil (*Hadroplontus litura*) demonstrated too many environmental restrictions to be an effective biocontrol for *C. arvense* (Zwölfer and Harris 1966). Release of *Rhinocyllus conicus*, a capitula-feeding beetle, affected *C. arvense* populations, but was considered ineffective due to the ability of the weed to reproduce readily through rhizomatous underground growth (Cripps et al. 2011). The final two insects released as biocontrol, *Lema cyanella* and *Urophora cardui*, were both similarly ineffective (Harris and Shorthouse 1996; Peschken et al. 1982). Recent developments in the epiphytotics of systemic infection of *C. arvense* using the rust fungus *Puccinia punctiformis* has been suggested as a potentially viable method of control (Berner et al. 2015; Berner et al. 2013). *Puccinia punctiformis* has not yet been established at lethal levels at field scales or in field conditions. However, research is underway to evaluate the management of *C. arvense* with *P. punctiformis* when the fungus is combined mechanical controls. Biological control of *C. arvense* has shown moderate success in greenhouse and laboratory studies but requires further research to be applicable for widespread adoption in United States agriculture (Cripps et al. 2011; Orloff et al. 2018).

Research Objectives

The overarching goal of this research was to assess the efficacy of contrasting integrated weed management treatments for perennial rhizomatous weeds in the organic cropping systems of the NGP. Specifically, our objectives were to determine how *C. arvensis* and *C. arvense* populations, density, and biomass responded to a spectrum of both tillage intensity and crop competition, with tillage intensity inversely related to crop competition.

Research chapters two and three focus on these objectives for each weed species. Chapter two evaluates the response of *C. arvensis* populations. The study took place at the Western Agricultural Research Center (WARC) in Corvallis, Montana from the fall of 2017 to harvest of 2020. Ten cropping system treatments were created and compared to be representative of the range of crop sequences and tillage methods available to small-grain farmers in Montana. Treatments were arranged on a spectrum of decreasing tillage intensity and increasing crop competition. For example, a treatment with two consecutive years of tilled fallow constituted maximum tillage and minimal crop competition, whereas a treatment with two consecutive years of perennial alfalfa forage constituted maximum crop competition and minimal tillage. Treatments in the middle of this gradient included annual and biennial monocot and dicot crops with minimal to moderate tillage. The overall objective of this experiment was to evaluate if cropping system treatments effected *C. arvensis* populations. An additional objective was to demonstrate how soil chemical and physical characteristics were affected by different treatments. We had three hypotheses: 1) Tilled fallow would decrease population growth of *C. arvensis* and decrease soil health; 2) both conventional and organic alfalfa treatments would also show decreases in *C. arvensis* populations; 3) cropping treatments that combined annual crops with reduced tillage or grazing would show increases in *C. arvensis* populations.

For the third chapter, we again analyzed both objectives but in relation to *C. arvense*. The experiment was conducted at two research farms associated with Montana State University: Central Agricultural Research Center (CARC) in Moccasin, Montana and the Fort Ellis Research and Education Farm (FE) in Bozeman, Montana. The study has completed three of four total years. Eight crop sequences and two tillage systems were compared. The eight crop sequences

spanned a range of fallow, annual, biennial, and perennial crop systems, while tillage systems were divided into a reduced (grazing and non-inversion) or standard (inversion) tillage systems. Cropping sequences were arranged on a spectrum of decreasing tillage intensity and increasing crop competition, with perennial alfalfa representing maximal forage crop competition and tilled fallow representing maximal tillage intensity. It was hypothesized that: 1) both tilled fallow and perennial forage would maintain the lowest *C. arvense* densities; 2) contrarily, it was also hypothesized that annual crop-focused sequences would see the greatest *C. arvense* densities; and 3) the reduced tillage system was hypothesized to have significantly greater *C. arvense* populations compared to the standard tillage system.

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CHAPTER TWO

CROP SEQUENCE AND CULTIVATION EFFECTS ON *CONVOLVULUS ARVENSIS* POPULATION DYANMICS IN SMALL GRAIN CROPPING SYSTEMS

Introduction

Weeds are difficult to manage in organic crop systems and can greatly reduce yields (Gomiero et al. 2011; McBride et al. 2015). Perennial, rhizomatous forbs, including *Convolvulus arvensis* L. Scop. (field bindweed), have been particularly problematic for organic producers (Orloff et al. 2018; Tautges et al., 2017_B). *Convolvulus arvensis* can reproduce through both seeds and vegetative shoots. The rhizomatous root system of *C. arvensis* is extensive and allows it to form dense patches in annual small grain cropping systems. Because *C. arvensis* reestablishes easily from root fragments, cultivation can physically spread the weed across agricultural landscapes (Sosnoskie et al. 2020; Weaver and Riley, 1982). *Convolvulus arvensis* seeds can also remain viable in soil for up to two decades (Weaver and Riley 1982). The prolific nature of the root system, long-lived seeds, and competitive growth directly contribute to *C. arvensis* weediness in both conventional and organic agricultural systems.

Historically, *C. arvensis* management in agroecosystems involved frequent tillage and soil cultivation practices to disrupt its perennial root system (reviewed by Sosnoskie et al. 2020). In modern conventional cropping systems, *C. arvensis* seedlings and aboveground shoots are temporarily reduced with chemical herbicides, though long-term reduction of established populations requires repeated herbicide applications (Bayat and Zargar 2020; Davis et al. 2018). In organic cropping systems, the use of systemic herbicides for suppressing perennial weeds is

not allowed. This has led to a reliance on recurrent mechanical cultivation for *C. arvensis* suppression. Intensive and repeated cultivation has reduced or eradicated *C. arvensis* in past studies, but cultivation has negative consequences for soil, including increased erosion potential, water loss, a decrease in soil stability, impacts to microbial communities, and a decrease in organic matter (Carretta et al. 2021; Kasper et al. 2009; Moraru and Teodor 2010; Seitz et al. 2019; Soane et al. 2012).

The negative consequences of intensive soil cultivation and tillage have led to a search for weed management that combines multiple tactics into an integrated weed management plan (Kassam et al. 2010; Larson et al. 2021; Lehnhoff et al. 2017). In organic systems, integrated weed management incorporates mechanical, biological, and cultural practices to suppress weeds (Benaragama and Shirtliffe 2013; Carr et al. 2012; Lehnhoff et al. 2017). Integrated weed management can provide a more sustainable approach to crop production by reducing the reliance on any single weed management practice. In organic, small grain cropping systems, integrated weed management is often focused on the combination of diverse competitive crop rotations and timely mechanical practices or sometimes livestock grazing to remove weed biomass.

Crop rotation is an essential cultural tactic for weed management. Rotations can include a diverse set of crops with different traits such as life cycle or growth habit, which is important for maximizing crop competition. For example, biomass and residue from competitive cover crops has been shown to suppress germination and growth of early-season weeds, specifically annual species, as well as allow for more frequent mechanical management (Flood and Entz 2018; Teasdale 1996). However, annual cover crops may be less competitive with rhizomatous

perennial weeds. Perennial forage crops, such as alfalfa (*Medicago sativa* L.), are more competitive with perennial weeds like *C. arvensis* (Tautges et al. 2017$_A$). The deep-reaching root systems allow for perennial crops to compete directly with *C. arvensis* for resources (e.g., water, soil nutrients).

In water-limited, semi-arid small grain cropping systems the adoption of cover or forage crops can be challenging due to their soil water use, depression of subsequent cash crop yields, and low biomass production which inhibits the suppression of weeds (Carr et al. 2021; DuPre et al. 2021; Unger and Vigil 1998). In studies conducted in small grain cropping systems vegetative mulch from cover crops failed to reduce established perennial weed populations (Carr et al. 2012). Similarly, while integrating livestock grazing into organic cropping systems to remove crop and weed biomass can be an effective way to reduce tillage and provide forage, it has also demonstrated problematic increases in both annual and perennial weed biomass when compared to conventional-no till and organic-tilled systems (Larson et al. 2021). Subsequent increases in weed density can reduce crop yield (Osterholz et al. 2020; Zikeli and Gruber 2017).

To improve integrated weed management of *C. arvensis* we compared ten cropping system treatments for three growing seasons to determine which treatments limited the growth of *C. arvensis*. Treatments were arranged on a spectrum of both tillage and crop competition. Tillage intensity was inversely related to crop competition. For example, the treatments ranged from a high soil disturbance system with two consecutive years of tilled fallow to a minimal soil disturbance system with two consecutive years of perennial alfalfa. Treatments in the middle of this spectrum had crop rotations with monocot and dicot annual and biennial crops and more moderate tillage intensities. We expected intensively tilled fallow and perennial forage to limit

the spread of *C. arvensis* more than combinations of annual crops and tillage. Repeated and frequent cultivation weakens *C. arvensis* rhizomes, preventing patch expansion and keeping root density low. Perennial alfalfa similarly limits *C. arvensis* density through direct plant competition. *Convolvulus arvensis* density was expected to be greatest in treatments with annual crops and minimal to moderate tillage because crop competition was weaker and tillage too infrequent to reduce populations.

Methods

Site Description

The field trial was conducted from the autumn of 2017 to the summer of 2020 at the Montana State University Western Agricultural Research Center in Corvallis, MT (46.3274° N, 114.0841° W, 3,600'). The experiment was conducted on a sandy-loam soil in the frigid Typic Haplustoll class, consisting of very deep, well-drained materials formed in alluvium and colluvium. Average annual precipitation for the area is 461 mm, with the majority falling between May and August (Western Regional Climate Center, 2023). A previous crop rotation study comparing crop-weed competition was conducted in the field from the spring of 2015 to the late summer of 2017 and organic certification was maintained for the last two years of the study (Miller, 2017, unpublished).

Experimental Design

The study used a randomized complete block design with three replicate blocks. Blocks were separated by 6 m buffer strips. Within each block, ten cropping system treatments were randomly assigned to 6 x 12 m plots. Treatment plots were separated by 1 m buffer strips. In

2015, 48 *Convolvulus arvense* rhizomes were transplanted into each plot. Rhizomes were between 15 and 20 cm in length.

Cropping System Treatments

Each of the ten cropping system treatments combined different crop species and different amounts of mechanical tillage (Tables 2.1 and 2.2). The cropping system treatments were arranged on a gradient of decreasing tillage intensity and increasing crop competition. Each cropping system treatment lasted three full growing seasons, starting either in 2017 with a fall-planted crop or in 2018 with a spring-planted crop. The maximum amount of soil disturbance occurred in two treatments that were kept in fallow for two years (Tillage 1, Tillage 2). In Tillage 1 and Tillage 2, mechanical tillage occurred every two to three weeks after initial *C. arvensis* emergence, usually in late March. Tillage 1 and Tillage 2 also had the lowest amount of crop competition by utilizing two consecutive years of fallow.

Five cropping system treatments had annual crop species, including mixtures of grain crops (e.g., spring wheat [*Triticum turanicum* Jakubz.], green manure cover crops (e.g., field pea [*Pisum sativum* L.], and tillage intensities. Of this group, the Wheat-CC treatment is a standard farmer practice in the semi-arid wheat-growing areas of North America (Cochran et al., 2006), and had three total tillage events annually including a pre-plant, a post-harvest, and a final fall disking. Other treatments in this group included Spring Till, Wheat-CC + till, Rye-CC + till, and Reduced Till and had types of crops and tillage timings. The Grazing-CC had increased crop competition by using a biennial sweet clover, and reduction in tillage by using livestock grazing to terminate forage. The remaining two treatments used a two-year perennial alfalfa rotation (Conventional Alfalfa, Organic Alfalfa), which constituted maximum competition and no soil

disruption. In 2020, all ten treatments were planted with Khorasan spring wheat (*Triticum turanicum* Jakubz.; Kamut ®). Seeding rates can be found in Table 1.

In total, four different field implements were used among the treatments for mechanical control of *C. arvensis*: a moldboard plow, a rototiller, a disc, and a flail mower (for cover crop termination). The moldboard plow and rototiller were both used in Tillage 1. The rototiller had a 15 cm maximum depth and was applied during the growing season, starting two weeks after *C. arvensis* emergence. The moldboard plow had a 25 cm depth and was used in early fall. A disc was used in other treatments requiring tillage at a 15 cm maximum depth. Cover crops were terminated with the flail mower and then incorporated with the disc. Conventional Alfalfa had two initial glyphosate applications (GlyStar® Plus; 2017: 1121 g ai ha^{-1}; 2018: 3365 g ai ha^{-1}). The Grazing-CC treatment used sheep grazing to terminate a biennial forage crop in the second year of growth. Further details on field actions can be found in Table 2.

Table 2.1. Crop species, seeding year, and seeding rate (kg ha^{-1}) for the ten cropping system treatments.

Treatment	Crop	Scientific name	Year	Seed rate, kg ha^{-1}
1. Tillage 1	Fallow (no crop)	N/A	2018, 2019	N/A
2. Tillage 2	Fallow (no crop)	N/A	2018, 2019	N/A
3. Wheat-CC[1] + till	Spring wheat	*Triticum turanicum* Jakubz.	2018	224
	Spring field pea	*Pisum sativum* L.	2019	112
4. Wheat-CC[1]	Spring wheat	*Triticum turanicum* Jakubz.	2018	224
	Spring field pea	*Pisum sativum* L.	2019	112
5. Spring till	Sorghum-sudangrass	*Sorghum bicolor x var. sudanese* L.	2018, 2019	11
	Faba bean	*Vicia faba* L.	2018, 2019	22
	Chickling vetch	*Lathyrus sativus* L.	2018, 2019	22
6. Rye-CC[1] + till	Winter rye	*Secale cereale L.*	2017, 2018	112
	Winter field pea	*Pisum sativum* L.	2018, 2019	112
7. Reduced till	Winter field pea	*Pisum sativum* L.	2017	56
	Winter rye	*Secale cereale L.*	2017	56
	Sorghum-sudangrass	*Sorghum bicolor x var. sudanese* L.	2018, 2019	11
	Faba bean	*Vicia faba* L.	2018, 2019	22
	Chickling vetch	*Lathyrus sativus* L.	2018, 2019	22
8. Grazing-CC[1]	Winter rye	*Secale cereale L.*	2017	112
	Yellow sweet clover	*Melilotus officinalis* L.	2018, 2019	5.6
9. Conventional alfalfa	Alfalfa	*Medicago sativa L.*	2018, 2019	29
	Common oat	*Avena sativa* L.	2018, 2019	45
10. Organic alfalfa	Alfalfa	*Medicago sativa L.*	2018, 2019	29
	Common oat	*Avena sativa* L.	2018, 2019	45
All treatments (1-10)	Spring wheat	*Triticum turanicum* Jakubz.	2020	160

Table 2.2. Timeline of management for ten cropping system treatments, including crop duration, tillage events, grazing, herbicide applications, and harvesting or termination.

Treatment ID	Field Actions	2017			2018												2019												2020					
		O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
1. Tillage 1	Crop	Fallow																													Wheat					
	Mechanical actions[1]						RT	RT	RT*	RT	RT	RT*									RT	RT	RT	RT	MP		M				D, CP					H
2. Tillage 2	Crop	Fallow																													Wheat					
	Mechanical actions[1]						D	RT	RT*	RT	RT	RT*									RT	RT	D	D	D		M				D, CP					H
3. Wheat-CC + till	Crop								Wheat													Spring pea									Wheat					
	Mechanical actions[1]	D					D		CP			H, D	D*							D, CP		D	D*	D*	D*						D, CP					H
4. Wheat-CC	Crop								Wheat													Spring pea									Wheat					
	Mechanical actions[1]						D		CP			H, D	D							D, CP		D	D	D	D						D, CP					H
5. Spring till	Crop									Cover crop mix												Cover crop mix									Wheat					
	Mechanical actions[1]						D		CP			D	D								D, CP			FM, D						D, CP					H
6. Rye-CC + till	Crop	Rye											Winter pea																		Wheat					
	Mechanical actions[1]												D									FM, D	D*	D*	D*						D, CP					H
7. Reduced till	Crop	Pea-rye mix								Cover crop mix												Cover crop mix									Wheat					
	Mechanical actions[1]								D			D	D											FM						D, CP					H
8. Grazing-CC	Crop	Rye											Sweet clover																		Wheat					
	Mechanical actions[1]												D									G	G								D, CP					H
9. Conventional alfalf	Crop									Alfalfa																					Wheat					
	Mechanical actions[1]	S							S		R	H										H		H	H						D, CP					H
10. Organic alfalfa	Crop									Alfalfa																					Wheat					
	Mechanical actions[1]						D		CP		R	H										H		H	H						D, CP					H

D – disking (* indicating two events in one month), FM – flail mow, G – grazing, H – harvest, M – compost manure addition, R – reseed, RT – rototill (* indicating two events in one month), S – herbicide spray

Sampling *C. arvensis* Populations

Convolvulus arvensis ramet counts were initiated in the summer of 2017, prior to fall crop planting. Subsequent ramet counts were conducted annually in each plot before crop termination or harvest (either July or August). *Convolvulus arvensis* ramets were counted inside the core of the plot (4 x 10 m). We excluded a 1 m buffer around the plot to avoid edge effects. In 2017, 2019, and 2020, in addition to density, four 0.5 x 0.5 m frames were randomly placed parallel to crop rows in the core of the plot. All biomass was cut, separated into *C. arvensis*, crop, and other weed biomass. The biomass was then dried and weighed. In the final year of the cropping treatments, wheat grain yield in each plot was estimated from a combine mounted yield monitor. Yield estimates were calculated from a 17 m^2 area in the center of the plot.

Soil Sampling and Testing

Soil was sampled from each plot twice, once in 2019 for aggregate stability testing, and once before planting spring wheat in 2020 for soil nutrient analysis. Both samples consisted of soil taken from 15 to 20 1-cm diameter soil cores that were composited and homogenized. A slake test was performed in 2019 to measure the stability of soil when exposed to rapid wetting (USDA, 1999). In 2020, soil was resampled from all plots and sent to Agvise Laboratories (Northwood, ND, USA) for soil physical and chemical analysis, which included analyses of P, K, Zn, Ca, Mg, N, CEC, organic matter, and pH. Soils were also analyzed for microaggregate, macroaggregate, and large macroaggregate percentages.

Statistical Analysis

We assessed changes in *C. arvensis* ramet density in each plot annually over the four-year period, beginning with an initial count in 2017 before treatments started and ending at harvest in 2020. We analyzed density using a linear mixed effects model (`lmer` in R-package 'lme4'; Bates et al., 2020). The model had the fixed effects of year, cropping system treatment, and the interaction between the two variables. Block and a unique plot identifier were added as random effects to account for grouping of replicate blocks and the repeated measures of the same plots over time. *Convolvulus arvensis* ramet density was scaled to ramets per m^2 and log transformed. Type-III ANOVA (`anova` in R-package 'lmerTest'; Kuznetsova et al. 2017) was used to determine if year, treatment, or their interaction accounted for variation in *C. arvensis* densities. Estimated marginal means of *C. arvensis* ramet density were calculated and compared using the Tukey post-hoc (`emmeans` in the R-package 'emmeans'; Lenth 2021). We estimated marginal and conditional R^2 using the `r.squaredGLMM` function in 'MuMIn' package (Bartoń 2023). The marginal R^2 describes proportionally the variance explained by the fixed effects, whereas the conditional R^2 describes the variance explained by both fixed and random effects (Nakagawa & Schielzeth 2013). We correlated density and biomass of *C. arvensis* in plots using density and biomass data from 2017, 2019, and 2020.

Interannual rates of ramet density change were calculated using the common growth rate equation [relative growth rate = $\ln(N_{t+1} / N_t)$], where N is the density of ramets in a plot, and t is an annual time step (Vandermeer and Goldberg, 2013). We fit a linear mixed effects model with transition period (e.g., 2017 to 2018) and cropping system treatment as the fixed effects, and plot and block as the random effects. Estimated marginal means were calculated and compared using the Tukey post-hoc method (`emmeans` in the R-package 'emmeans'; Lenth 2021). To

determine if growth rates were positive or negative between transition periods, the estimated marginal means were tested against the default of zero (relative growth rate = 0) using a reference grid and `test` function in the R-package "emmeans" (Lenth 2021).

To assess if soil chemical characteristics differed among treatments, we tested individual soil parameters (Supplemental Table 1.3) using linear mixed effects models with treatment as a fixed effect and block as a random effect. To assess soil stability, slake test data were scored as integer counts and analyzed using a generalized linear model with a Poisson distribution (Carpenter and Chong, 2010). Post-hoc Tukey comparisons using the 'emmeans' package (Lenth, 2021) were conducted to assess differences in soil aggregate stability between treatments.

Linear mixed effects models were assessed visually for normality and homoscedasticity. Differences between all explanatory and response variables for all models were analyzed at the $P < 0.05$ level. All statistical analyses and graphics were performed and produced in the statistical environment R, version 4.0.2 (R Core Team 2020; graphics produced using R-packages "ggplot2" (Wickham, 2016) and "cowplot" (Wilke, 2019)).

Results

Convolvulus arvensis Density Changes

The interaction between year and cropping system treatment affected *C. arvensis* ramet density (Table 2.3; Figure 2.1) Fixed effects explained 42% ($R^2 = 0.42$) of variation, and fixed and random effects accounted for 71% ($R^2 = 0.71$). There were no differences in *C. arvensis* ramet density among cropping system treatments in the first year of the study (Supplemental Table 1.1). There was also no difference among cropping system treatments in the second year of

the study, 2018 (Supplemental Table 1.1). In the first two years, mean ramet density ranged between 0.3 and 1.3 plants per meter squared (2017 and 2018; Figure 2.1; Supplemental Table 1.1). In the third year ramet density differed among treatments (Figure 2.1; Supplemental Table 1.1). Six cropping system treatments had ramet densities that ranged between 0 and 1 per m^2, these included Tillage 1 (0.01 ± 0.01 SE ramets/m^2), Tillage 2 (0.1 ± 0.1), Conventional Alfalfa (0.2 ± 0.2), Organic Alfalfa (0.09 ± 0.09), Spring Till (0.4 ± 0.4), and the Reduced Till (1.0 ± 1.0). The Wheat-CC + till (P = 0.005; 4.0 ± 4.0), Grazing-CC (P = 0.01; 3.0 ± 3.0), Rye-CC + till (P = 0.01; 2.8 ± 2.8), and Wheat-CC (P = 0.01; 2.5 ± 2.5) had the greatest ramet densities in the third year, significantly greater than Tillage 1 (Supplemental Table 1.1).

In the final year of the study (2020), Tillage 1 (0.01 ± 0.01) had lower mean *C. arvensis* density than the Reduced Till treatment (1.7 ± 1.7), Rye-CC + till treatment (2.8 ± 2.8), the Wheat-CC + till treatment (5.1 ± 5.0), and the Grazing-CC treatment (6.7 ± 6.7; Figure 2.1; Supplemental Table 1.1). The Conventional Alfalfa treatment (0.04 ± 0.04) also had lower density than the Grazing-CC treatment (P = 0.02) and the Reduced Till treatment (P = 0.03). At the end of the study, treatments with the highest mean *C. arvensis* density had annual broadleaf cover crop in one or two years, especially in the Grazed-CC and Rye-CC + till treatment. In contrast, the treatments with the lowest density had perennial alfalfa or frequent tillage during the growing season.

The interannual relative growth rates of *Convolvulus arvensis* varied in response to cropping system treatment (P = 0.04), and the interaction of treatment and transition period (P = 0.005; Table 2.4). Over the course of the study, the relative growth rate of *C. arvensis* density was negative in the Tillage 1 treatment (P < 0.01; Figure 2.2a). Over the same period, the

Grazing-CC had a positive relative growth rate of *C. arvensis* density ($P < 0.01$; Figure 2.2a). The average annual relative growth rate of the remaining eight treatments did not significantly differ from zero over the course of the study (Figure 2.2a). However, the relative growth rate of *C. arvensis* did differ among cropping systems within year. For example, the Organic Alfalfa and the Reduced Till treatments had relative growth rates that did not differ from zero over the course of the study, but *C. arvensis* density was much lower in the Organic Alfalfa (0.4 ramets per m^2) when compared to the Reduced Tillage plots (1.7 ramets per m^2; Supplemental Table 1.1).

The relative growth rate of *Convolvulus arvensis* rate in the Reduced Till plots was negative during the first transition (relative growth rate =-1.64) but increased to (relative growth rate =2.24) from the first to the second transition (from 2017-2018 to 2018-2019; Figure 2.2c). In the Reduced Till treatment, the *C. arvensis* population growth rate increase coincided with a reduction in tillage and the second season of a broadleaf cover crop mix. During the same period, *C. arvensis* rates of change in the Tillage 1 plots decreased (relative growth rate =-3.54), in conjunction with the start of intensive, consistent mechanical soil disruption (from 2017-2018 to 2018-2019; Figure 2.2c). *Convolvulus arvensis* relative growth rate was also negative (relative growth rate = -1.95) in the Organic Alfalfa treatment during the second transition period (from 2017-2018 to 2018-2019; Figure 2.2c), which corresponded to the second year of perennial crop growth. Finally, the Wheat-CC treatment had *C. arvensis* growth rates decrease from the second to third transition period (from 2018-2019 to 2019-2020; Figure 2.2d), which may be due in part to the planting of a more competitive wheat grain crop instead of a broadleaf annual crop.

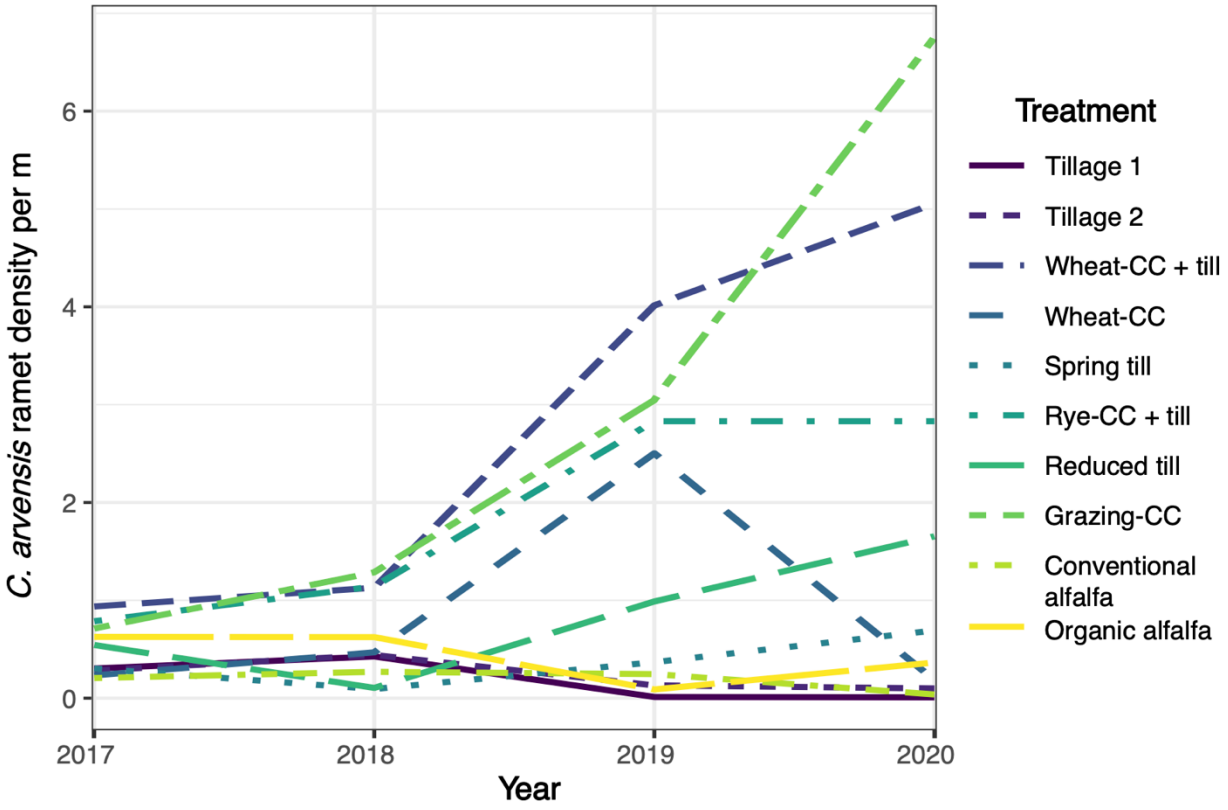


Figure 2.1. *Convolvulus arvensis* estimated means for ramet density per m^2 across the ten cropping system treatments over a four-year period (2017-2020), assessed at Corvallis, MT, USA, as derived from the underlying linear mixed-effects model.

Table 2.3. Analysis of variance (ANOVA) for a linear mixed effects model of Convolvulus arvensis density on the log scale as effected by year (2017-2020), cropping system treatment, and their interaction. Model was analyzed at $P < 0.05$.

Predictor	Sum Sq.	Mean Sq.	NumDF	DenDF	F-value	Pr(>F)
Year	0.5	0.2	3	60	0.21	0.95
Treatment	32.1	3.6	9	18	1.80	0.06
Year: Treatment	108.0	4.0	27	80	2.63	**<0.001*** **

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

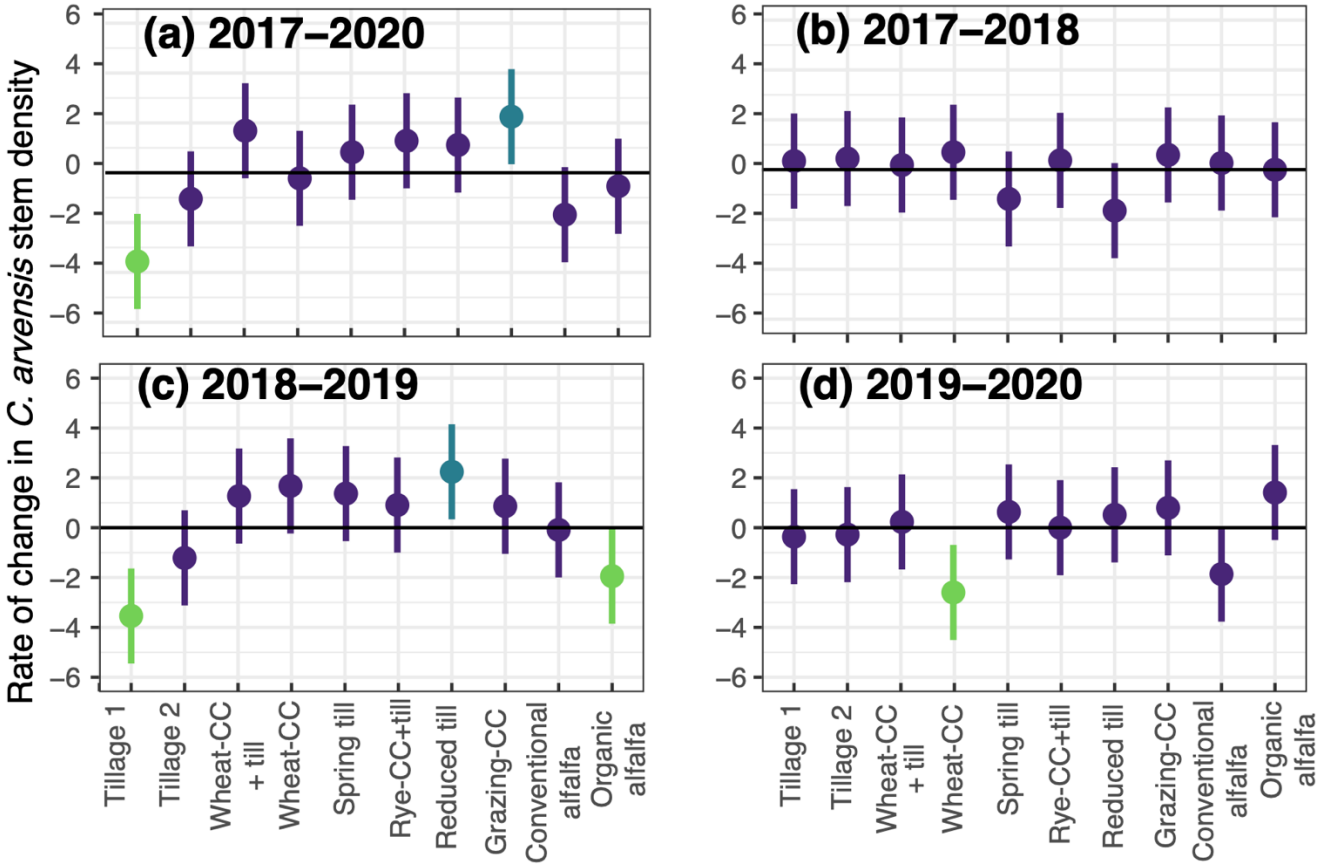


Figure 2.2. *Convolvulus arvensis* mean relative growth rate (change in ramet density over time) with 95% confidence intervals for relative population growth rates by cropping system treatment, as derived from the underlying linear mixed-effects model for 2017 to 2018 **(a)**, 2018 to 2019 **(b)**, 2019 to 2020 **(c)**, and over the entirety of the study period from 2017 to 2020 **(d)**. Green lines indicate a significant decrease in relative growth (e.g., significantly less than zero) over the given time interval ($P < 0.05$). Blue lines indicate a significant increase in relative growth (e.g., significantly greater than zero) over the time interval ($P < 0.05$). Purple lines indicate relative growth rate does not differ from zero.

Table 2.4. Analysis of variance (ANOVA) of a linear mixed effects model of *Convolvulus arvensis* relative population growth rates in response to transition period (e.g., 2017-2018), cropping system treatment, and their interaction. Model was analyzed at $P < 0.05$.

Predictor	Sum Sq.	Mean Sq.	NumDF	DenDF	F-value	Pr(>F)
Transition period	1.4	0.5	3	60	0.2	**0.04***
Treatment	56.1	6.2	9	20	2.6	0.90
Transition period: Treatment	148.4	5.5	27	60	2.3	**0.005****

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

Biomass, Wheat Yield, and Soil Analyses

Over the course of the study, estimated *C. arvensis* biomass was significantly correlated with plot density (cor = 0.72). In the final study year (2020), wheat yields did not vary by treatment ($P = 0.3$; Supplemental Table S3). Soil aggregate percentages and most soil chemical properties did not vary by treatment (Supplemental Tables 1.4 – 1.7). Soil stability did vary by cropping system treatment ($P < 0.001$; Figure 2.3). The Tillage 1 treatment had lower soil stability compared to all other treatments ($P < 0.001$), except the Tillage 2 treatment (Figure 2.3). Tillage 1 had a mean score of 3.6, indicating that 50% of the structural integrity of the sample was lost 30 to 300 seconds after wetting, or less than 10% of soil remained on the sieve after five wetting cycles (USDA 1999). Tillage 2 differed from all treatments (4.0; $P = 0.001$) except the Spring Till treatment and the Wheat-CC + till treatment. Most treatments had mean scores of 5 or higher, indicating that 25% to 75% of soil remained on the testing sieve after five wetting cycles (USDA 1999).

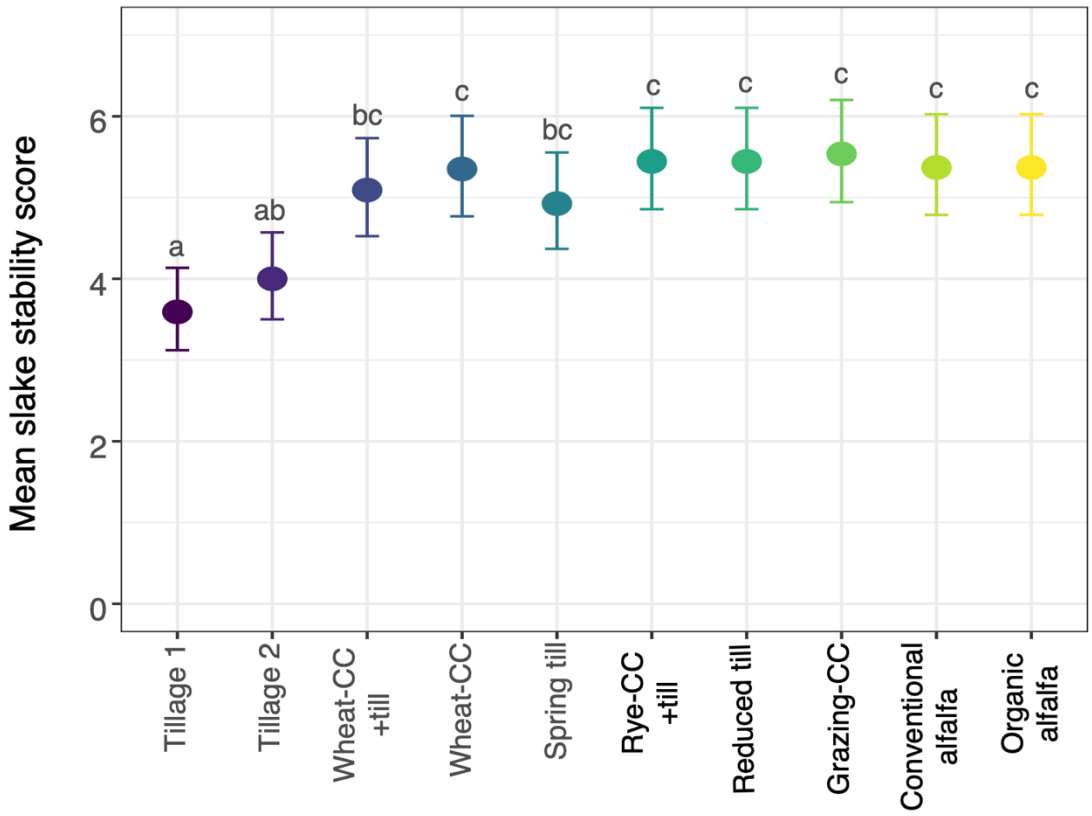


Figure 2.3. Comparison of aggregate soil stability across ten cropping system treatments. Letters indicate significant post-hoc differences between soil stability in the cropping system treatments ($P < 0.05$).

Discussion

Perennial rhizomatous weeds in organic, small grain cropping systems are difficult to manage (DeDecker et al. 2014; OAEC 2013; Orloff et al. 2018; Tautges et al., 2017_B). In this experiment, we recorded increases in mean *C. arvensis* density in certain annual and biennial crop rotations with minimal to moderate tillage. By the fourth growing season, *C. arvensis* mean densities were largest in the Grazing-CC treatment, which incorporated livestock grazing and a biennial sweet clover crop. Extensively tilled fallow plots (Tillage 1, Tillage 2) and alfalfa forage (Conventional Alfalfa, Organic Alfalfa) plots had consistently low mean *C. arvensis* densities. Tilled fallow decreased *C. arvensis* density over the course of the study. However, tilled

treatments also had lower soil aggregate stability than other treatments, and therefore a greater erosion potential. Our research suggests incorporation of periods of perennial crops or extensively tilled fallow may maintain lower *C. arvensis* populations in organic cropping systems, while prolonged use of annual crops in rotation or large reduction in tillage can allow for expansion for *C. arvensis* populations.

Annual cropping systems in this study had more varied changes in *C. arvensis* density than tilled fallow or perennial forage cropping system treatments. Following an initial lag in density changes, our study found *C. arvensis* densities increased in both annual and biennial cropping systems in years where a broadleaf annual crop was sown. For example, the standard wheat cover crop rotation (Wheat-CC) had low *C. arvensis* density in the initial wheat phase, which then increased in the field pea phase and decreased during the final spring wheat phase. Annual cover crops vary in their ability to suppress weeds in dryland agroecosystems and have produced mixed results depending on crop type (Kumar et al. 2020). Cereals tend to be more competitive with perennial weeds than broadleaf annuals due to increased residue cover (Derksen et al. 2002; Ghimire et al. 2018; Lensenn et al. 2015). Biennial sweet clover (*Melilotus officinalis* L.), especially when undersown with other crops, has suppressed perennial weeds in dryland cropping systems due to high biomass production (Blackshaw et al. 2001; Schoofs & Entz 1999). However, research in Montana demonstrated the integration of a sweet clover cover crop and sheep grazing to produce high weed density and low subsequent wheat yields (Larson et al. 2021). In our study, the cropping system treatment with biennial sweet clover had one of the highest densities of *C. arvensis*. In the initial establishment phase during the first year of the biennial life cycle, the competitive ability of sweet clover is limited, though in both years the

density of *C. arvensis* increased. Because some annual crops have limited competitive ability and tillage can only be used in certain stages annual cropping systems are likely more suspectable to increases in *C. arvensis* and maybe more variable.

In comparison to the annual crop rotations, *C. arvensis* density did not increase in perennial alfalfa treatments. In a review of *C. arvensis* distribution in the United States, Boldt et al. (1998) reported increased *C. arvensis* infestations after land was removed from the Conservation Reserve Program, suggesting the transition from perennial cover to annual crops allowed for spread of the more competitive perennial *C. arvensis*. Meiss et al. (2010) similarly reported perennial crop fields to have lower perennial weed populations (specifically *C. arvensis*) than annual crop fields. Our study demonstrates that perennial crops were associated with lower *C. arvensis* populations.

The competitive ability of alfalfa with perennial cropland weeds has been well-documented (Favrelière et al. 2020; Meiss et al. 2010; Tautges et al. 2017$_A$), but in this paper we show that it can be used successfully for weed management in organic cropping systems. Integrating an alfalfa phase into conventional small grain cropping system can suppress perennial weeds better than continuous cereal crops (Ominski et al. 1999). In organic systems specifically, Grosse et al. (2021) demonstrated that the choice of a perennial alfalfa-grass crop rotation was the most important factor for managing perennial weeds. Our results suggest that incorporating competitive perennial crops, particularly alfalfa, can effectively prevent the growth of *C. arvensis* populations.

The success of the intensive tillage-fallow treatment confirms the results of historical studies, which saw reduced or even eradicated *C. arvensis* populations using thorough and

repeated mechanical disturbance (as reviewed by Sosnoskie et al. 2020). In the dryland, organic cropping systems of the Northern Great Plains, soil deterioration due to increased mechanical cultivation is a threat. Though we found similar soil chemical properties among the cropping system treatments, physical soil stability was significantly lower in the intensively tilled fallow after only two years of repeated cultivation. While successful in decreasing *C. arvensis* populations, the adverse effects of such extensive tillage have made it impractical and unsustainable for wide-scale weed management. Alternatively, while no-tillage or reduced tillage is less damaging for soil stability, it has been shown to increase perennial weed presence due to insufficient reduction of underground rhizomes (Buhler et al. 2002; Hume et al. 1991; Menalled et al. 2001).

Overall, we demonstrated that annual crop-dominated systems can lead to increases in *C. arvensis* populations, especially in livestock-integrated treatments, cropping systems with reduced tillage, and treatments with phases of broadleaf forage crops. Intensively tilled fallow reduced *C. arvensis* populations but was also associated with low soil aggregate stability. Integrating consecutive years of alfalfa into grain production suppressed *C. arvensis* populations as well as intensive tillage. To improve the sustainability of weed management in dryland organic grain systems, incorporation of a perennial ley phase is recommended to both suppress perennial weeds and reduce dependence on mechanical cultivation.

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CHAPTER THREE

CROP SEQUENCE AND TILLAGE EFFECTS ON *CIRSIUM ARVENSE* POPULATIONS IN DRYLAND ORGANIC SYSTEMS

Introduction

Modern conventional agriculture relies on chemical inputs to maintain simplified ecosystems for the purpose of reducing weed pressure and increasing crop yields. Organic agricultural systems, which cannot employ synthetic herbicides, generally support a more robust ecosystem with a greater diversity and abundance of species, including weedy species, when compared to conventional agricultural systems (Belde et al. 2000; Bengtsson et al. 2005; Berbeć et al. 2020). The decision to forgo external inputs like synthetic herbicides and fertilizers in organic agricultural settings results in 20% to 34% lower average yields when compared to conventional production (de Ponti et al. 2012; Kniss et al. 2016; Seufert et al. 2012). Depressed crop yields and lack of consistent control tactics have led weed management to be ranked as a top research concern for organic farmers (OAEC 2013; Lotter 2001; Tautges et al. 2017).

The types of weed species that occur in conventional or organic fields depend not only on the use of chemical herbicides, but also upon other agronomic practices such as crop selection, crop rotations, and tillage method (Ball 1992; Blackshaw et al. 1994; Shrestha et al. 2002; Menalled et al. 2001). In temperate areas where wheat (*Triticum aestivum* L.) is grown, perennial weeds are disproportionally represented in organic systems compared to conventionally managed systems (Davies and Welsh 2002; Gallandt 2014; Rydberg and Milberg 2000). Worldwide, rhizomatous *Cirsium arvense* L. Scop. (Canada, creeping, or Californian thistle) is one of the

most difficult to manage perennial weed species in temperate cropping areas (Moore 1975; Pimentel et al. 2000; Tiley 2010). Across the Northern Great Plains (NGP) of the United States, the weed is of particular concern to organic wheat growers (Donald and Khan 1992; Entz et al. 2000; OAEC 2013; Tautges et al. 2017). As a perennial forb, *C. arvense* grows and spreads readily through vegetative propagation (Donald 1990; Moore 1975). Buds form quickly on horizontal roots and create new, adventitious shoots, allowing for rapid lateral growth (Donald 1990; Moore 1975). The vertical roots of *C. arvense* commonly grow as deep as 2 m with lateral roots spreading equally far in a growing season (as reviewed by Moore 1975; Donald 1990). In agroecosystems, viable root fragments of the weed can be moved through tillage (Štolcová 2002). *Cirsium arvense* can resprout from root pieces as small as 5 to 7 cm, with regeneration from root fragments as small as 10 mm observed in a greenhouse setting (Gustavsson 1997; Hamdoun 1972). In addition to vegetative spread, *C. arvense* can be a prolific seeder, producing anywhere from 600 to 6,000 viable seeds per stem (Amor & Harris 1974; Bakker 1960; Bostock & Benton 1979; van Leeuwen 1987). The ability of *C. arvense* to propagate vegetatively, coupled with a high seed production, create challenges in attempts to suppress this weed species.

One strategy suggested to counteract increased weed pressure in organic agriculture is to use integrated weed management (IWM). IWM is the application of multiple weed management techniques, including biological, chemical, cultural, and mechanical controls (Blackshaw et al. 2008; Liebman et al. 1997; Swanton & Weise 1991). Specifically, IWM has also been suggested to minimize the impact of *C. arvense* in organic agricultural productions in the NGP (Davis et al. 2018; Orloff et al. 2018). However, little research on the suppression of *C. arvense* using IWM tactics has been documented (Orloff et al. 2018). For many organic producers in the NGP,

repeated mechanical cultivation is the primary tactic for suppression of *C. arvense* and other problematic perennial weeds (McErlich and Boydston 2013; OAEC 2013). Within tilled systems, *Cirsium arvense* populations decline in response to increasing amounts of soil cultivation (Dierauer & Lefebvre 2022; Graglia et al. 2006; Hodgson 1958; Tiley 2010). However, soil erosion and water loss due to intensive cultivation make the option untenable for long-term management of *C. arvense* in semi-arid, dryland cropping regions like the U.S. NGP (Carr et al. 2011; Tanaka et al. 2007).

In the NGP, reduced- and no-tillage systems often have soils with higher organic carbon, greater microbial biomass and abundance, greater soil aggregate stability, and faster infiltration rates with less erosion when compared to standard tillage systems (Helgason et al. 2009; Liebig et al. 2004; Peterson et al. 1998). However, the use of reduced tillage methods generally results in higher weed biomass with a higher prevalence of perennial weeds in organic systems (Halde et al. 2015; Hofmeijer et al. 2019; Larson et al. 2021; Zikeli & Gruber 2017). In organic zero-tillage systems, Carr et al. (2013) concluded that established perennial weeds were not suppressed by cover crop mulch. Surveys of organic grain producers in the NGP have also reiterated that zero-tillage systems are not widely used (OAEC 2013; Tautges et al. 2017). Grazing with livestock such as sheep (*Ovie aries*) is proposed in place of tillage to remove cover crop and weed biomass (Popay and Field 1996), but limited studies on crop-livestock integration indicate mixed success in suppressing weeds (Barroso et al. 2015; Larson et al. 2021; Lehnhoff et al. 2017).

Cultural weed management via diverse and competitive crops and crop sequences is often coupled with mechanical tactics into IWM plans for organic producers (Liebman et al.

1997). Annual crops common in the NGP's organic small-grain systems vary in ability to compete with deeper-rooted *C. arvense.* Cereal crops compete better with perennial weeds than broadleaf annuals (Derksen et al. 2002; Ghimire et al. 2018; Lensenn et al. 2015). Deep-rooted biennial (e.g., sweet clover [*Melilotus officinalis* L.]), perennial broadleaf forages (e.g., alfalfa [*Medicago sativa* L.]) and perennial grassland-pastures (Favrelière et al. 2020; Ominski et al. 1999; Melander et al. 2016) are better competitors with *C. arvense* than annual crop species. While successful in suppressing perennial weeds like *C. arvense*, deep-rooted crops or temporary pastures can severely deplete soil moisture levels and depress subsequent grain yields in dryland agricultural systems (Entz et al. 2002; Miller et al. 2019). Thus, it is crucial to understand the potential trade-offs of diversified integrated weed management strategies in dryland organic agricultural productions.

To evaluate the effect of integrated management strategies on *C. arvense* populations in dryland organic cropping systems, eight cropping sequences with an inverse gradient of cropping and tillage intensity were compared in a three-year study at two sites in Montana, USA. Cropping sequences were arranged on a spectrum of crop competition, with competition defined by total days of crop growth in the field. Two tillage systems, standard and reduced, were applied to crop sequences. The reduced tillage system generally limited soil disturbance to non-inversion methods and was infrequent. Our hypotheses were: 1) *C. arvense* populations would response negatively to crop sequences that featured perennial forage crop competition or tilled fallow; 2) *C. arvense* populations would increase with consecutive years of annual crops and fewer total days of crop competition; and 3) *C. arvense* populations would be greater in the reduced tillage system compared to the standard tillage system.

Methods

Site Descriptions

The study was conducted at two Montana State University research locations: the Central Agricultural Research Center (CARC) (47°03’33.8’N, 109°56’53.5’W; 1275 m) near Moccasin and the Ft. Ellis Research Farm (FE) (45°40’02.9’N, 110°58’40.4’W; 1469 m) in Bozeman, MT. The Bozeman site receives an average of 507 mm of precipitation annually and has soils with an available water holding capacity of 20 cm, whereas the Moccasin site receives an average of 391 mm of precipitation and has soils with an available water holding capacity of 13 cm (Davis Soil Lab 2023; PRISM Climate Group 2023). The Bozeman site is a Blackmore silt loam (fine-silty, mixed, superactive, frigid Typic Argiustolls) and the Moccasin site is a Danvers-Judith clay loam (fine, smectitic, frigid Vertic Argiustolls and fine-loamy, carbonatic, frigid Typic Calciustolls; UC-Davis Soil Lab 2023; USDA 2013). Both sites have a mean annual temperature range of 6.5 to 6.6°C (PRISM Climate Group 2023). Further information on precipitation and temperature at study sites for the duration of the experiment can be found in Supplemental Table S3.1.

Experimental Design

Eight crop sequences and two tillage systems were compared at both locations. The treatments were arranged in a randomized complete block design, as an eight-by-two factorial at CARC and in a split-plot design with a completely random arrangement at FE. At CARC, there were four complete blocks with 16 plots (inclusive of each combination of eight crop sequences and two tillage systems) in each block for a total of 64 plots. Plot dimensions were 6 m (20 ft) in width and 9.1 m (30 ft) in length. Adjacent plots within a block were separated by a 3-m (10 ft)

buffer planted to alfalfa (*Medicago sativa* L.), with 15 m (50 ft) wide alleys planted to alfalfa between each block.

To better facilitate grazing at the Ft. Ellis Research Farm (FE) for the reduced tillage treatment, tillage systems were randomly assigned to six fenced whole plots (15 m [50 ft] N-S x 90 m [295 ft] E-W) and crop sequences randomly assigned to split plots (5.5 m [18 ft] N-S x 15 m [50 ft] E-W) within whole plots. There were three whole plots receiving standard tillage and three receiving reduced tillage, for a total of 48 split plots. Adjacent plots were separated by a 3 m (N-S) or 7.5 m (E-W) tilled buffer.

Tillage Methods

Two tillage methods were compared in the study. At FE, the standard tillage method consisted of soil inversion with a chisel plow and a reduced tillage method, which used either inversion tillage at 50% frequency compared to the standard tillage system or a wide sweep with no soil inversion. In standard tillage at CARC, plots were disked with a 1.8 m tandem disk at a 12.7 cm depth. Secondary tillage consisted of cultivation with a .8 m tool bar with S-tine shanks mounted with 30 cm sweeps at a 9 cm depth. In reduced tillage at CARC, plots were undercut with a 1.5 m blade plow that sometimes was preceded with a light disking to enable soil penetration using the blade plow, both at a depth of 7.6 cm. Grazing with sheep was used to terminate all non-grain crops in the reduced tillage plots, coinciding with crop termination in the standard tillage plots. All plots at CARC were mowed and then cultivated with a 4.5 m field cultivator with 35.5 cm S-tine sweeps in the first study year prior to seeding of crops.

At the FE location, when opportunity presented, tillage was performed with field scale equipment across entire or partial blocks in both tillage systems, with tillage operations in the

reduced tillage plots set at 50% of standard tillage plots. Primary spring tillage was conducted with a 4.4 m wide chisel plough equipped with 39 cm wide soil-inverting shovels at a depth of 10 to 15 cm, with rear-mounted tine harrows set in a relaxed position to avoid moving plant residues. Secondary tillage for final seedbed preparation was conducted with a 4.3 m wide vibra-shank field cultivator with 18 cm wide shovels at a depth of 10 to 15 cm without harrows. Primary post-harvest fall tillage was either conducted with the chisel plough or a 4.5 m disk harrow with 51 cm notched disks on the front gang and 53 cm smooth disks on the rear gang was used to the depth of field moisture (7-10 cm) to chop crop stubbles in place, prior to chisel plough. In-season tillage was conducted by plot for cover crop termination and fallow maintenance with 2.4 m wide toolbar with eight, 37 cm wide soil inverting shovels at a 15 cm soil depth in standard tillage plots. In the reduced tillage plots, the same tool bar was used either with 50% frequency for cover crop termination or with three, 85 cm wide non-inversion blades at a depth of 10 cm for fallow maintenance. A forage harvester was used at FE to terminate all non-grain crops in the standard tillage plots. Grazing with sheep was used to terminate forage crops in the reduced tillage plots, coinciding with crop termination in the standard tillage plots. The timing of tillage events at FE and CARC can be found in Table 3.1.

Crop Sequences

Eight, four-year crop sequences were compared in the study (Table 3.2). All crop sequences were not represented in each year, but rather sequentially across the four-year study as was done in similar research (e.g., Halde et al. 2015; Tiemann et al. 2015). Crop sequences were arranged across a spectrum of crop use (forage vs. grain), crop competitiveness, and soil disturbance. Sequences included crops that are widely grown by organic farmers (e.g., spring

wheat) or are of interest (e.g., hemp [*Cannabis sativa* L.]) in Montana and the broader NGP. In the final year of the study, all treatments were (CARC) or will be (FE) planted to spring wheat at a rate of 80 kg ha^{-1} (240 live kernels m^{-2}) to make yield comparisons. This will occur at FE in 2023 and is excluded from this thesis. Seeding rates can be found in Table 3.2.

Crop competitiveness was inversely organized relative to tillage frequency in the sequences. Competitiveness was broadly defined by days of crop growth in the field and was considered a competitive characteristic given the perennial nature of *C. arvense* (Table 3.3). For annual crops, days of competition were calculated for each year at both sites using the total days in the field from seeding date to termination date. For perennial alfalfa, seeding date in the first year was used as the start of days of competition, with final termination used as the absolute end date. Start of days of competition for perennial alfalfa in years after establishment were estimated using a conservative start date of 1 May for the Bozeman location and 24 April for the Moccasin location, where the climate generally puts crop development one to two weeks earlier than the Bozeman location. End day of competition for perennial alfalfa outside of final year termination were estimated using hard "killing" frost (low daily temperature at or below -7°C) in the fall (P. Miller, personal communication, 2023). Biennial sweet clover estimates were calculated using the same metrics as alfalfa. Temperature data for both sites were derived from the NOWData database (National Oceanic and Atmospheric Administration 2023).

The first crop sequence ("Alfalfa") was used to provide maximum competition with perennial *C. arvense*. The alfalfa treatment had an average of over 400 cumulative days of direct competition with *C. arvense* over the first three years of the study (Table 3.3). Additionally, haying or grazing of alfalfa once to twice a season, usually mid-summer (mid- to late June) and

late summer, when possible (mid-August), would stunt *C. arvense* growth. The second crop sequence ("SW-cereal forage") was an annual monocot counterpart to perennial broadleaf alfalfa. Winter triticale (x *Triticosecale* Wittm.) followed by a millet crop (*Setaria italica* L.) was expected to provide roughly 360 total days of direct crop competition over the first three study years. However, because summer-planted millet did not germinate either year at either site due to lack of precipitation, the realized competitive value of millet was zero, decreasing the overall competitiveness of this sequence to 230 cumulative days over three years. Again, haying or grazing of triticale mid-summer was timed to act as a detriment to *C. arvense* growth.

The third ("SW-lentil-pea") and fourth ("Lentil-SW-pea") sequences were similar with biennial sweet clover (*Melilotus officinalis* L.) planted with a grain crop and established in the second year to provide increased competitive pressure on *C. arvense* in the third year. The presence of biennial sweet clover would have resulted in around 360 total days of competition over the first three years (Table 3.3). Unfortunately, due to damage from sweet clover weevil (*Sitona cylindricollis* Fahr.), no sweet clover crop survived in either sequence and was replaced with a much less competitive legume: annual Austrian winter pea (*Pisum sativum* L.). This replacement reduced the overall competitiveness of the third and fourth sequences to 263 days of total crop competition over three years.

The fifth sequence ("SW-double forage") incorporated both monocot (wheat and barley [*Hordeum vulgare* L.]) and dicot (field pea) annual crops with mid-summer haying or grazing to disrupt *C. arvense* growth. The cumulative days of direct crop competition for the fifth sequence was 240 days over the three-year study period (Table 3.3). Termination of forage crops mid-summer would disrupt *C. arvense* populations generally two to four weeks earlier than a grain

harvest. The sixth sequence ("Lentil-pea-hemp") was designed so biennial sweet clover planted with a less competitive lentil crop in the first year competed with *C. arvense* in the second year before following with a hemp grain crop in the third year. This sequence totaled more than 330 days of crop competition over three years (Table 3.3). However, sweet clover did not survive due to weevil feeding and was replaced with Austrian winter pea in the second year, reducing the overall competitive value of the sequence to 274 days of direct competition over three years.

The seventh sequence ("CCM-SW") used a highly diverse nine species cover crop mix ("CCM") grown in the first and third year and wheat ("SW") in the second year which allowed periodic tillage subsequent to mid-summer cover crop termination. In this sequence, the total days of competition over the first three years was 234 days (Table 3.3). Finally, the eighth sequence ("SW-double fallow") constituted a tillage check where a year of wheat was followed by two consecutive years of high-frequency (every 14 to 21 days) tilled fallow, resulting in only 124 days of direct plant competition over the first three study years (Table 3.3).

Table 3.1. Technical operations and sampling periods at both experimental field sites (Central Agricultural Research Center, Moccasin, MT, USA; Ft. Ellis Research Farm, Bozeman, MT, USA).

Treatment ID	Field Actions	Y2																				Y3												Y4							
		M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
1. Alfalfa	Crop	Barley				Alf.								Alfalfa											Alfalfa											Wheat						
	Mechanical actions[1]					F									H		H										H/T			T^2											H
2. SW-cereal forage	Crop	Triticale												Triticale			Millet[3]								Triticale				Millet[3]								Wheat						
	Mechanical actions[1]			H												H/T	T											H/T		T^2											H
3. SW-lentil-pea	Crop	Wheat												Lentil											Winter pea											Wheat						
	Mechanical actions[1]					H											H/T											H/T		T^2											H
4. Lentil-SW-pea	Crop	Lentil												Wheat											Winter pea											Wheat						
	Mechanical actions[1]					H											H/T											H/T		T^2											H
5. SW-double forage	Crop	Wheat												Barley-pea											Winter pea											Wheat						
	Mechanical actions[1]					H										H	T											H/T		T^2											H
6. Lentil-pea-hemp	Crop	Lentil-SC												Pea											Hemp											Wheat						
	Mechanical actions[1]			M		T										H	T												H	T^2											H
7. CCM-SW	Crop	CCM												Wheat											CCM											Wheat						
	Mechanical actions[1]			H		T											H/T											H/T		T^2											H
8. SW-double fallow	Crop	Wheat					Fallow																													Wheat						
	Mechanical actions[1]					H								T	T	T^2	T	T	T							T	T^2	T^2		T^2											H

[1] "F" – fall seeding; "H" – harvest (forage crops harvested with forage harvest, mowed, or grazed with livestock; grain crops harvested with combine); "T" – tillage event (T^2 indicates two events in same month)

[3] Millet did not germinate but was seeded both years.

Table 3.2. Crop species and seeding rate (kg ha^{-1}) for the eight crop sequences.

ID Name	Plant species	Scientific name	Seed rate, kg ha^{-1}
1. Alfalfa	Barley	*Hordeum vulgare* L.	62
	Alfalfa	*Medicago sativa L.*	13.5
2. Forage grains	Spring wheat	*Triticum aestivum* L. emend. Thell.	80
	Triticale	x *Triticosecale* Wittm.	102
	Foxtail millet	*Setaria italica* L.	5.6
3. SW-lentil-pea	Spring wheat	*Triticum turanicum* L.	80
	Lentil	*Lens culinaris* L.	41.5
	Sweet clover	*Melilotus officinalis* L.	5.6
	Spring pea	*Pisum sativum* L.	135
4. Lentil-SW-pea	Lentil	*Lens culinaris* L.	67.3
	Spring wheat	*Triticum aestivum* L. emend. Thell.	80
	Sweet clover	*Melilotus officinalis* L.	5.6
	Spring pea	*Pisum sativum* L.	135
5. SW-double forage	Spring wheat	*Triticum aestivum* L. emend. Thell.	80
	Barley	*Hordeum vulgare* L.	62
	Winter pea	*Pisum sativum* L.	142
6. Lentil-pea-hemp	Lentil	*Lens culinaris* L.	67.3
	Sweet clover	*Melilotus officinalis* L.	5.6
	Spring pea	*Pisum sativum* L.	135
	Hemp	*Cannabis sativa* ssp. *sativa* L.	45
7. CCM-SW	Spring wheat	*Triticum aestivum* L. emend. Thell.	80
	Barley	*Hordeum vulgare* L.	6.7
	Emmer	*Triticum dicoccon* S.	14.6
	Oats	*Avena sativa* L.	7.8
	Pea	*Pisum sativum* L.	43.7
	Lentil	*Lens culinaris* L.	7.8
	Faba bean	*Vicia faba* L.	51.6
	Yellow mustard	*Sinapis alba* L.	1.1
	Radish	*Raphanus sativus* L.	5.6
	Turnip	*Brassica rapa* ssp. *rapa L.*	1.1
8. SW-fallow	Spring wheat	*Triticum aestivum* L. emend. Thell.	80
	Tilled fallow	N/A	N/A

Table 3.3. Total days of growth for crops in each cropping sequence at both study sites over the three-year study period. "Actual" total days of crop growth is reflective of realized days of crop growth. "Predicted" total days is an estimate of crop growth if all experimental crops had established in every year.

Central Agricultural Research Center, Moccasin, MT					
	Y1 (2019)	**Y2 (2020)**	**Y3 (2021)**	**Actual**	**Predicted**
Growth start date[a]	----------	24-Apr	24-Apr	--------	----------
Last frost[b]	18-May	23-May	22-May	--------	----------
First frost	28-Sep	8-Sep	17-Sep	--------	----------
First killing frost[c]	1-Oct	17-Oct	----------	--------	----------
Consecutive frost-free days	133	108	118	359	----------
Crop sequence					
1. Alfalfa[d]	74 (+ 65)	176	76	391	391
2. SW-cereal forage	132	63 **(+ 51[e])**	44 **(+ 70[e])**	239	360
3. SW-lentil-pea	132	84 **(+ 68[f])**	44 **(76[f])**	260	360
4. Lentil-SW-pea	116	98 **(+ 54[f])**	44 **(76[f])**	258	344
5. SW-double forage	132	63	44	239	239
6. Lentil-pea-hemp	116 **(+ 24[f])**	63 **(87[f])**	103	282	330
7. CCM-SW	75	98	72	245	245
8. SW-double fallow	132	0	0	132	132
Ft. Ellis Research Farm, Bozeman, MT					
	Y1 (2020)	**Y2 (2021)**	**Y3 (2022)**	**Actual**	**Predicted**
Growth start date[a]	----------	1-May	1-May	--------	----------
Last frost[b]	11-May	6-Jun	23-May	--------	----------
First frost	8-Sep	17-Sep	10-Sep	--------	----------
First killing frost[c]	21-Oct	7-Nov	----------	--------	----------
Consecutive frost-free days	120	103	110	333	----------
Crop sequence					
1. Alfalfa[d]	60 (+ 112)	190	58	420	420
2. SW-cereal forage	115	47 **(+ 88[e])**	60 **(+ 47[e])**	222	357
3. SW-lentil-pea	115	96 **(+ 88[f])**	60 **(85[f])**	271	384
4. Lentil-SW-pea	102	102 **(+ 83[f])**	60 **(85[f])**	264	355
5. SW-double forage	115	69	60	244	244
6. Lentil-pea-hemp	102 **(+ 71[f])**	69 **(74[f])**	95	266	342
7. CCM-SW	60	102	60	222	222
8. SW-double fallow	115	0	0	115	115

[a] Growth start date used to determine growing start dates for frost-hardy alfalfa [*Medicago sativa* L.] and sweet clover [*Melilotus officinalis* L.] after first establishment year.
[b] Last and first frosts determined by low temperature at or below 0°C (used as end date for frost-sensitive millet [*Setaria italica* L.])
[c] Killing frost (low temperature at or below 20°F) used to determine end date for frost-hardy alfalfa and sweet clover growth for a season
[d] Added value of barley nurse crop.
[e] Added anticipated value of millet crop had it successfully germinated and established.
[f] Added anticipated value of sweet clover crop had it successfully survived.

Sampling Methods

Six, 0.5 m^2 sampling frames were used to collect density and aboveground biomass samples from each treatment plot. Frame locations were stratified within each plot. Two, 0.5 m^2 sampling frames (1 m by 0.5 m dimensions) were randomly assigned a location in each third of the plot, avoiding a 1 m buffer around plot edges to minimize edge effects. Frames were placed perpendicular to crop rows to sample aboveground crop and weed density and biomass from three crop rows and four inter-row spaces. Sampling was performed with a handheld sickle and occurred just prior (within one week) to crop termination, grazing, or harvest in all treatments. All plant stems were cut at the soil surface and separated by crop (if present), target weed (*C. arvense*), and non-target weeds. Both crop and *C. arvense* density counts were taken concurrently with biomass sampling. Biomass samples were dried after sampling at approximately 45°C for >72 hours to a constant weight in the Montana State University Plant Growth Center (Bozeman, MT, USA) for estimation of dry matter yield.

Statistical Analyses

To assess the effects of tillage system, crop sequence, year of sequence (Years 1, 2, and 3), and their interactions on *C. arvense* ramet density, first 1 m^2 frames were averaged for each plot (CARC) or split plot (FE). The study sites were analyzed separately. Generalized linear mixed-effects models with a negative binomial distribution were used to model *C. arvense* density for both study sites (`glmer.nb` in R package 'lme4'; Bates et al. 2015). A negative binomial distribution was used to alleviate statistical issues with overdispersion due to excessive zero data and the wide range of *C. arvense* ramet counts in the dataset (Stoklosa et al. 2022). The model selection process is described in detail in Appendix S3.3. Tillage system, crop sequence,

year of sequence, and their interactions were considered fixed effects with block and unique plot identifiers considered random effects to account for repeated measures over time. A Type-III ANOVA was performed on the generalized linear mixed-effects models to determine if the explanatory variables accounted for differences in *C. arvense* density ('Anova' in R package 'car'; Fox et al. 2023).

Cirsium arvense biomass data were also aggregated from frame data to an average value for each plot (CARC) or split plot (FE). A linear mixed-effects model (`lmer` in R-package 'lme4'; Bates et al. 2020) was used to determine the effects of tillage system, crop sequence, year of sequence, and their interactions on *C. arvense* biomass. Fixed and random effects were retained from the previous model. *Cirsium arvense* biomass data were log-transformed to meet assumptions of normality and homogeneity. In the model-fitting process, a likelihood ratio test was used to determine best model fit and remove variables when necessary (`lrtest` in R-package 'lmtest'; Hothorn et al. 2022). A Type-III ANOVA (`anova` in R-package 'lmerTest'; Kuznetsova et al. 2017) was used to determine if any explanatory variables accounted for differences in *C. arvense* biomass over time. Estimated marginal means of *C. arvense* biomass were calculated and compared using Tukey post-hoc comparisons (`emmeans` in the R-package 'emmeans'; Lenth 2021).

Results

Weather

Weather varied considerably across years and sites in this study (Supplemental Table S3.1). At CARC, the first year of the study (2019) had the most favorable conditions, with growing season precipitation representing 112% of the long-term average. The following two

years (2020 and 2021) at CARC were drier than average. June and July of the third year (2021) had drought conditions, including above average temperatures and monthly precipitation that was 21% and 48%, respectively, of long-term averages. Conversely at FE, the first and second study years (2020 and 2021) had above average temperatures and lower monthly precipitation in the growing season. Conditions improved in the third year (2022) when growing season precipitation was 119% of the long-term average.

Central Agricultural Research Center

Cirsium arvense Density Changes There was no significant interaction between tillage method and all other variables for *C. arvense* density at CARC. An interaction between year and crop sequence was detected for *C. arvense* density ($P < 0.001$; Table 3.4). In the first and second study years, less *C. arvense* ramets were produced in the SW-double fallow sequence (0.04 ramets per $m^2 \pm 0.04$ [SE]; 0.1 ± 0.1) than the three highest-density sequences: the SW-lentil-pea (5.8 ± 4.9; 7.8 ± 6.5), the SW-double forage (8.3 ± 7.0; 5.4 ± 4.6), and the CCM-SW (5.5 ± 4.7; 10.7 ± 9.0). In the third year, the SW-double fallow sequence (0.4 ± 0.4) continued to have fewer *C. arvense* ramets than the SW-lentil-pea sequence (15.4 ± 12.9) and the CCM-SW sequence (13.6 ± 11.4; Figure 3.1).

Primarily, differences in *C. arvense* density occurred within sequences over time. *Cirsium arvense* density increased from the first to the second year only within the SW-cereal forage sequence (0.7 ± 0.6 to 4.0 ± 3.4; Figure 3.2). There were no changes in *C. arvense* density within crop sequences from the second to third study year. *Cirsium arvense* ramet density increased in five crop sequences from the first to third year: SW-cereal forage (0.6 ± 0.6 to 7.7 ± 6.6), SW-lentil-pea (5.8 ± 4.9 to 15.4 ± 12.9), lentil-SW-pea (1.3 ± 1.1 to 4.1 ± 3.5), CCM-SW

(5.5 ± 4.7 to 13.6 ± 11.4), and SW-double fallow (0.0 ± 0.0 to 0.4 ± 0.4; Figure 3.2). Four of the sequences, excluding SW-double fallow, consisted of three consecutive years of annual monocot or dicot crops, and all but the CCM-SW sequence were characterized by crop failure (either millet or sweet clover). Crop failures led to a reduction of > 100 days of crop competition with *C. arvense* (Table 3.3). Crop sequences with the lowest *C. arvense* densities in the third study year were the alfalfa (2.1 ± 1.8), lentil-pea-hemp (1.7 ± 1.5), and SW-double fallow sequences (0.4 ± 0.4; Figure 3.2). The alfalfa and SW-double fallow sequences were characterized by either the most days (> 400) of direct competition with *C. arvense* populations or by frequent mechanical disruption of soil (upwards of 15 tillage events) (Tables 3.1 and 3.3). The lentil-pea-hemp sequence did not have the greatest number of days of crop competition nor the most tillage events but did have the latest harvest date (10 August) for the third study year. The third growing season had drier than average conditions in the growing season and lead to severe desiccation of late-harvested hemp and *C. arvense* plants.

Table 3.4. *Cirsium arvense* estimated mean ramet density per m^2 at Central Agricultural Research Center (CARC; Moccasin, MT, USA) as effected by crop sequence, year of crop phase (Y1, Y2, etc.), tillage method, and their interactions as determined by an analysis of variance (ANOVA) for a generalized linear mixed effects model with a negative binomial distribution.

Fixed Effects	**Chisq**	**DF**	**p-value**
Crop sequence	12.8	7	0.08
Year of crop sequence	6.1	2	**0.05**
Tillage method	0.2	1	0.61
Tillage: crop sequence	8.7	7	0.27
Tillage: year	4.8	2	0.09
Crop sequence: year	34.4	14	**0.002**
Tillage: crop sequence: year	19.6	14	0.14

Note: ANOVA table using type III Wald chi-square test.

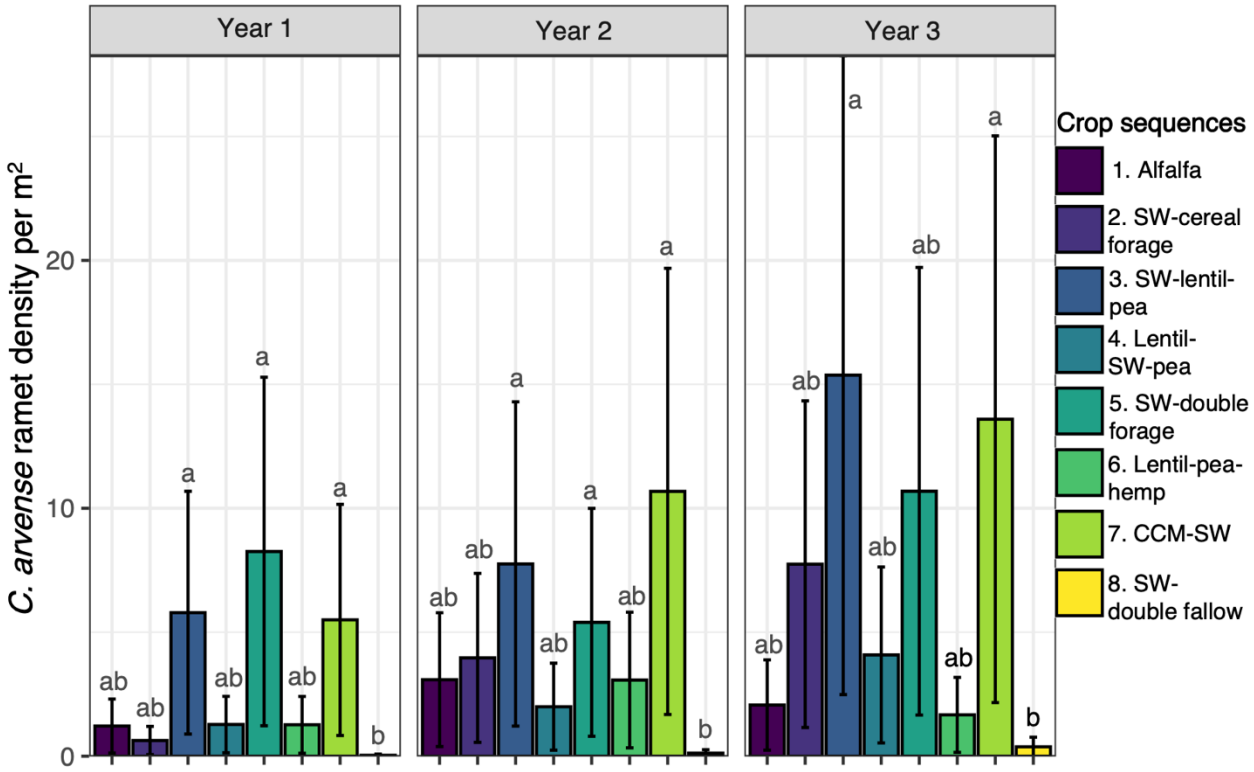


Figure 3.1. Differences in *C. arvense* estimated mean ramet density per m^2 among eight crop sequences at Central Agricultural Research Center (CARC; Moccasin, MT, USA) within years of the study. Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in *C. arvense* ramet density among crop sequences.

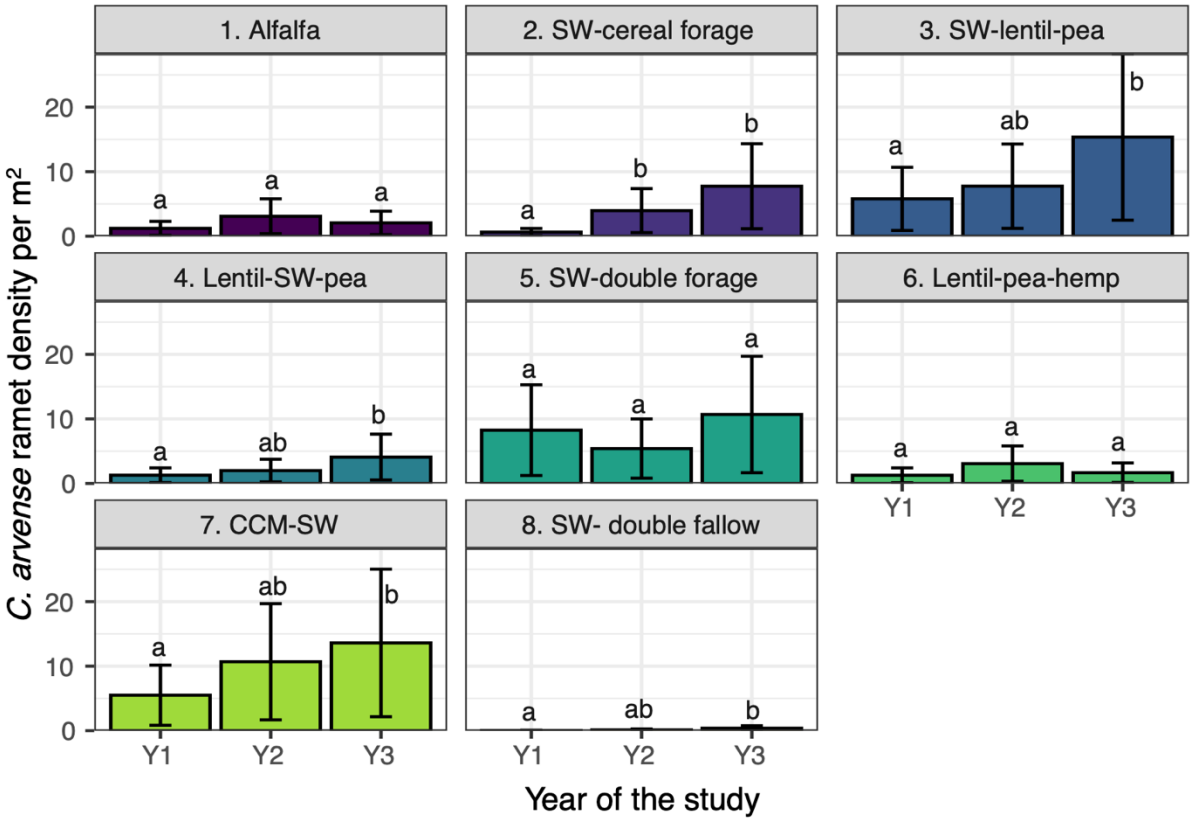


Figure 3.2. Differences in *C. arvense* estimated mean ramet density per m^2 within the eight crop sequences at Central Agricultural Research Center (CARC; Moccasin, MT, USA) across years of the study. Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in *C. arvense* density within a crop sequence.

<u>*Cirsium arvense* Biomass Changes</u> An interaction between year and crop sequence was detected for *C. arvense* biomass ($P < 0.05$; Table 3.5). In the first year, less *C. arvense* biomass was produced in the SW-double fallow sequence (0.1 ± 0.5 [SE] g per m^2) than the SW-lentil-pea (10.6 ± 8.5) and the SW-double forage sequences (12.1 ± 9.5; Figure 3.3). SW-double fallow (0.2 ± 0.5) continued to produce less *C. arvense* biomass than the SW-cereal forage (25.2 ± 19.5), SW-double forage (11.8 ± 9.3), and CCM-SW (27.5 ± 21.3; Figure 3.3) sequences in the second year, when there were between four and seven tillage events in the SW-double fallow sequence. Conversely, tillage was infrequent (e.g., before planting) in SW-cereal forage, SW-

double forage, and CCM-SW sequences and less competitive annual crops were planted in the second year. In the third year, less *C. arvense* biomass was produced in the SW-double fallow sequence (0.4 ± 0.7) than in the SW-lentil-pea (19.2 ± 14.9) and CCM-SW sequences (32.2 ± 24.8; Figure 3.3). The alfalfa sequence (1.3 ± 1.4) also produced less *C. arvense* biomass than the CCM-SW sequence in the third study year (Figure 3.3). CARC experienced severe drought conditions in the third growing season (Table S3.1) which could have contributed to the stagnation in *C. arvense* biomass from the second to third year among most crop sequences, even as *C. arvense* density increased.

Fewer differences in *C. arvense* biomass were detected within crop sequences over time. Only two crop sequences produced more *C. arvense* biomass at the end of the study than the beginning. More *C. arvense* biomass was produced in the third year than the first year in SW-cereal forage sequence (0.9 ± 1.0 to 11.9 ± 9.4) and the CCM-SW sequence (8.6 ± 6.9 to 32.2 ± 24.8; Figure 3.4). The SW-cereal forage sequence was characterized by two consecutive years of crop failure (millet), which reduced the total number of days of crop competition (< 240 days over the three-year study period; Table 3.3). The CCM-SW sequence had a similarly low number of days of crop competition with *C. arvense* (< 245 days over the three-year study period; Table 3.3).

Table 3.5. *Cirsium arvense* aggregated biomass (g per m^2) at Central Agricultural Research Center (CARC; Moccasin, MT, USA) as effected by crop sequence, year of crop phase (Y1, Y2, etc.), tillage method, and their interactions as determined by an analysis of variance (ANOVA) for a linear mixed effects model.

Fixed Effects	Numerator DF	Denominator DF	F statistic	p-value
Crop sequence	7	45	3.6	**0.004**
Year of crop sequence	2	96	5.1	**0.008**
Tillage method	1	45	0.8	0.38
Crop sequence: year	14	96	1.9	**0.04**
Crop sequence: tillage	7	45	0.8	0.57
Tillage: year	2	96	0.1	0.87
Crop sequence: tillage: year	14	96	1.2	0.27

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

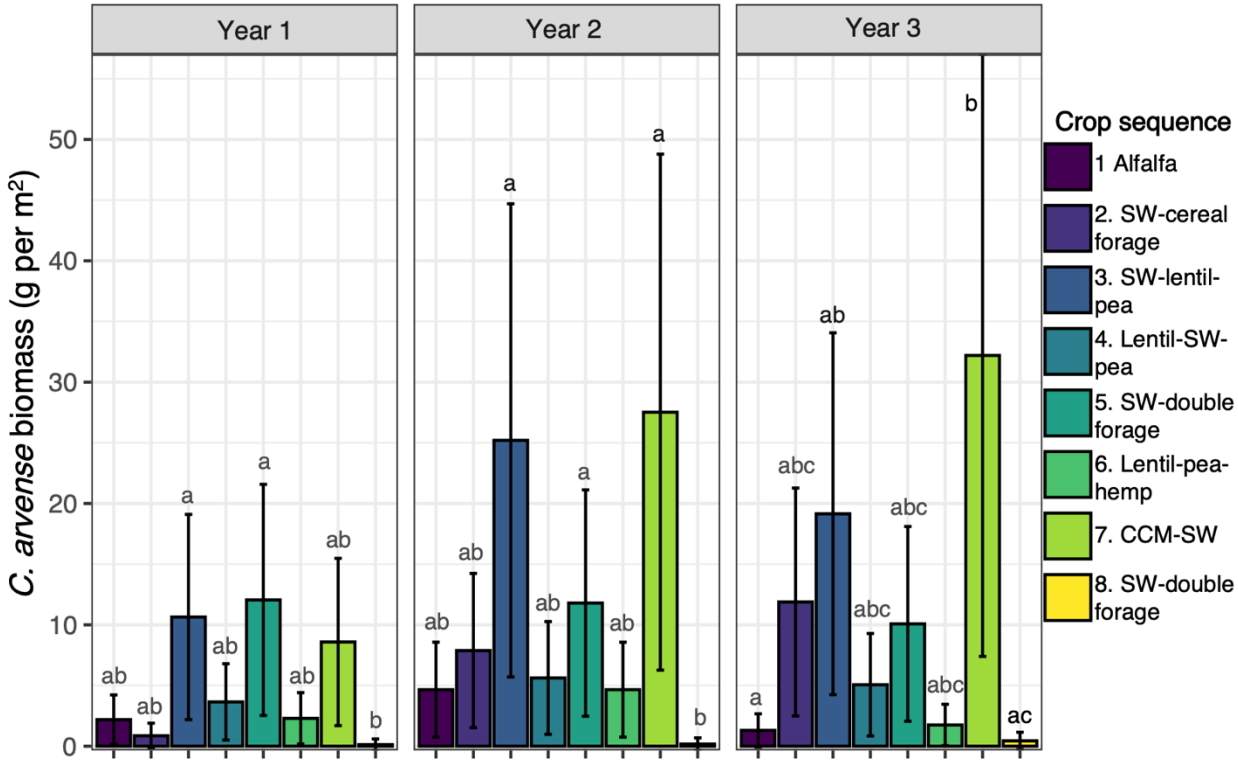


Figure 3.3. *Cirsium arvense* estimated mean biomass (g per m^2) among the eight crop sequences at Central Agricultural Research Center (CARC; Moccasin, MT, USA) within years of the study. Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in mean *C. arvense* biomass among crop sequences within year.

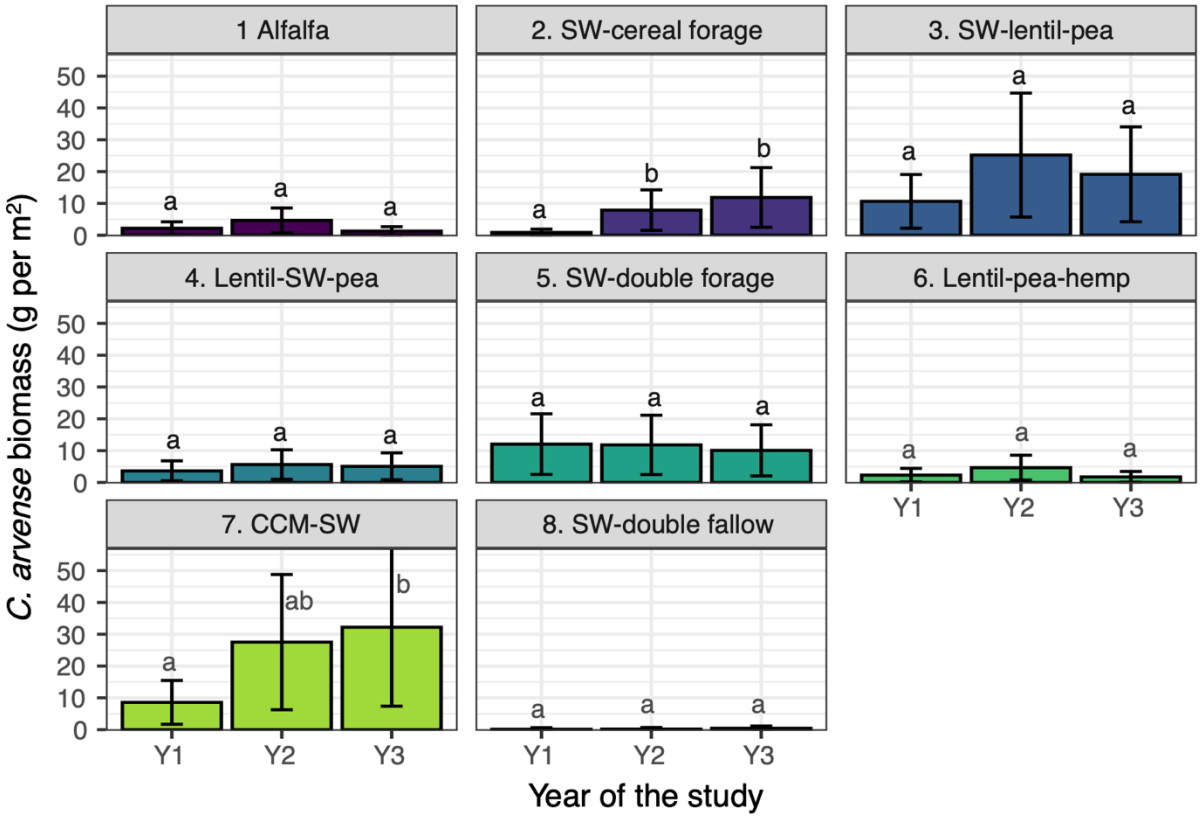


Figure 3.4. *Cirsium arvense* estimated mean biomass (g per m^2) within the eight crop sequences at Central Agricultural Research Center (CARC; Moccasin, MT, USA) across years of the study. Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in mean *C. arvense* biomass within a crop sequence between years.

Ft. Ellis Research Farm

Cirsium arvense Density Changes A three-way interaction among crop sequence, year, and tillage was detected for *C. arvense* density at FE (Table 3.6). *Cirsium arvense* density did not differ between tillage methods within crop sequences across years of the study. Among crop sequences, however, *C. arvense* ramet density had more differences within the standard tillage system than the reduced tillage system over time (Figure 3.5). Two-way interactions existed between crop sequence and year ($P < 0.001$) and crop sequence and tillage ($P = 0.01$) for *C. arvense* density at FE (Table 3.6). Post-hoc tests showed no differences in *C. arvense* density

between tillage methods within crop sequences. However, there were differences among crop sequences by tillage method and year, as is reflected in the three-way interaction (Figure 3.5).

At FE, the reduced tillage system had no differences among crop sequences over the first two years of the study (Figure 3.5). In the third study year, the alfalfa (0.4 ± 0.6) and the SW-double fallow sequences (0.6 ± 0.8) produced less *C. arvense* ramets than the SW-cereal forage sequence (17.3 ± 21.1) and the lentil-pea-hemp sequence (18.7 ± 24.1; Figure 3.5). The standard tillage system at FE had much greater variation in *C. arvense* density among sequences in first two years of the study. In the first study year, the alfalfa (0.4 ± 0.6), SW-lentil-pea (0.6 ± 0.7), and SW-double forage (0.2 ± 0.3) sequences all produced significantly less *C. arvense* ramets than the lentil-pea-hemp (16. ± 21.0) and SW-double fallow (18.1 ± 22.5) sequences (Figure 3.5). In the second study year, the alfalfa (1.5 ± 1.9) and the SW-double forage (0.2 ± 0.3) produced less *C. arvense* ramets than the lentil-pea-hemp sequence (34.9 ± 43.3; Figure 3.5). In the third study year, the alfalfa (0.3 ± 0.4) sequence produced less *C. arvense* ramets than the SW-cereal forage (14.6 ± 18.2), lentil-SW-pea (15.5 ± 19.6), lentil-pea-hemp (59.1 ± 73.3), and CCM-SW (12.3 ± 15.4) sequences (Figure 3.5). The SW-double forage sequence (0.5 ± 0.6) also produced less *C. arvense* ramets than the SW-cereal forage, lentil-SW-pea, and lentil-pea-hemp sequences (Figure 3.5). Finally, the SW-double fallow (2.6 ± 3.4) sequences also produced less *C. arvense* ramets than the lentil-pea-hemp sequence (Figure 3.5).

Across both standard and reduced tillage systems, the crop sequences with the greatest *C. arvense* densities at the end of the study were those composed of three consecutive years of annual crops. Additionally, high-density sequences included those with reduced competitive ability due to the failure of millet and sweet clover crops. Crop failures in millet and sweet clover

reduced the days of crop competition by more than 90 days in the SW-cereal forage, lentil-SW-pea, and lentil-pea-hemp sequences and therefore limiting the competitive potential of the sequences. Conversely, the alfalfa, which had over 400 days of direct competition with *C. arvense,* and the SW-fallow sequence, which had over 14 tillage events, maintained the lowest *C. arvense* densities over the study period when compared to other crop sequences (Tables 3.1 and 3.3).

There were also differences in *C. arvense* density within crop sequences separated by tillage method over time (Figure 3.6). General trends in *C. arvense* growth or reduction over time, however, were similar across tillage methods within a crop sequence. For example, the SW-cereal forage sequence produced more *C. arvense* ramets in the third year compared to the first year in both the standard and reduced tillage plots, but the rate of growth was quicker in reduced tillage (Figure 3.6). The exception to this trend was the SW-double forage sequence. In this sequence in the reduced tillage plots, *C. arvense* ramet density increased from the first to second year but remained constant through all three years in the standard tillage plots. Overall, nearly all crop sequences produced more *C. arvense* ramets in the third year than the first year, regardless of tillage method (Figure 3.6). In both reduced and standard tillage plots, *C. arvense* density in the alfalfa sequence was consistently low throughout the study interval (Figure 3.6). The SW-double fallow was the only sequence to produce less *C. arvense* ramets in the third year than the first year (11.2 ± 14.0 to 1.6 ± 2.1; Figure 3.6). Again, the sequences with the greatest number of days of competition (> 400 days in the alfalfa; Table 3.3) or greater number of tillage events (upwards of 16 events over three years in SW-double fallow; Table 3.1) produced the least *C. arvense* ramets at the culmination of the study.

Table 3.6. *Cirsium arvense* estimated mean ramet density per m^2 at Ft. Ellis Research Farm (FE; Bozeman, MT, USA) as effected by crop sequence, year of crop phase (Y1, Y2, etc.), tillage method, and their interactions as determined by an analysis of variance (ANOVA) for a generalized linear mixed effects model with a negative binomial distribution.

Fixed Effects	**Chisq**	**DF**	**p-value**
Crop sequence	13.4	7	0.06
Year of crop sequence	3.6	2	0.17
Tillage method	0.2	1	0.65
Tillage: crop sequence	16.9	7	**0.02**
Tillage: year	3.8	2	0.15
Crop sequence: year	198.1	14	**<0.001**
Tillage: crop sequence: year	38.5	14	**<0.001**

Note: ANOVA table using type III Wald chi-square test.

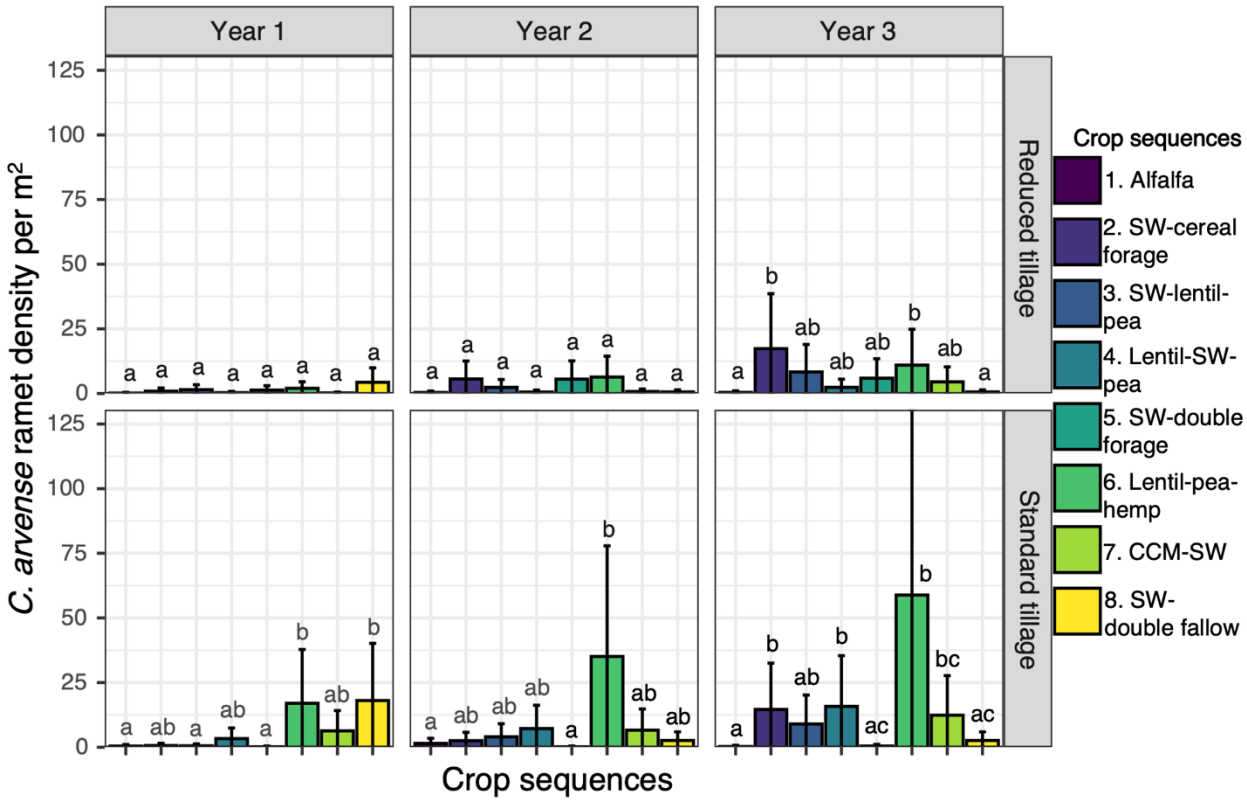


Figure 3.5. *Cirsium arvense* estimated mean ramet density per m^2 among crop sequences separated by tillage method over time at the Ft. Ellis Research Farm (FE; Bozeman, MT). Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in *C. arvense* ramet density among crop sequences.

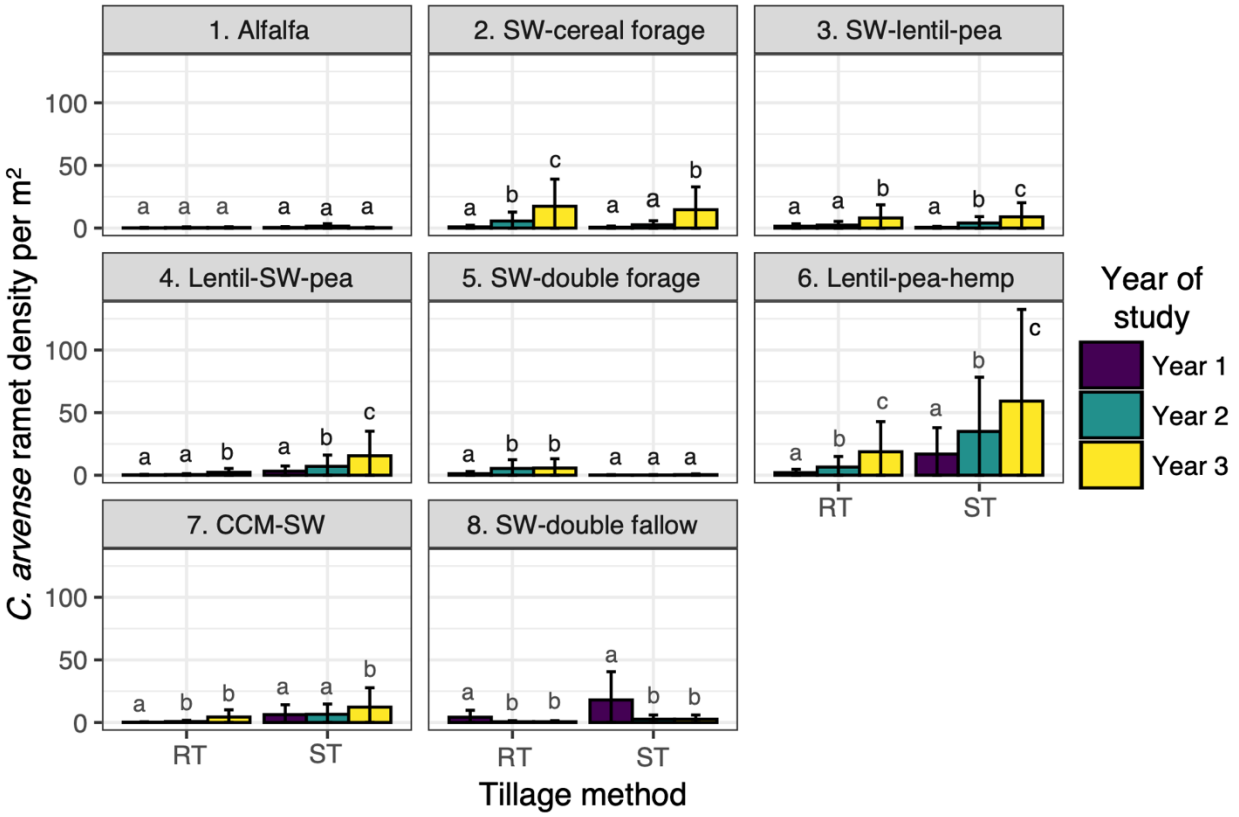


Figure 3.6. *Cirsium arvense* estimated mean ramet density per m^2 within crop sequences separated by tillage method over time at the Ft. Ellis Research Farm (FE; Bozeman, MT). Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in *C. arvense* ramet density within crop sequences.

<u>*Cirsium arvense* Biomass Changes</u> No three-way interaction between tillage method, crop sequence, and year was detected for *C. arvense* biomass at FE. There was an interaction between crop sequence and year for *C. arvense* biomass ($P < 0.001$; Table 3.7). In the first year, less *C. arvense* biomass was produced in the alfalfa (2.6 ± 2.9 [SE] g per m^2) and SW-cereal forage (1.8 ± 2.3) than the SW-double fallow sequence (48.2 ± 40.2; Figure 3.7). In the second year, less *C. arvense* biomass was produced in the alfalfa (2.5 ± 2.9) and SW-double fallow sequences (1.9 ± 2.4) than the lentil-pea-hemp sequence (62.3 ± 51.7; Figure 3.7).The alfalfa and SW-fallow had either more growing days in the field (approx. 115 to 200 days) or a much greater

frequency of mechanical tillage in the season (upwards of 6 events) compared to the lentil crop. In the third year of the study, less *C. arvense* biomass was produced in the alfalfa (10.48 ± 1.7) and SW-double fallow (12.84 ± 234.1) sequences compared to the SW-cereal forage sequence (43.9 ± 36.7) and the lentil-pea-hemp (93.0 ± 79.9; Figure 3.7). Overall, crop sequences with the greatest *C. arvense* biomass at the culmination of the study were composed primarily annual broadleaf species that have fewer days of direct competition with perennial *C. arvense* than alfalfa or less intensive or frequent tillage than SW-double fallow (Table 3.3).

Five crop sequences had changes in mean *C. arvense* biomass over the three-year study period at FE. The SW-fallow treatment had a significant decrease in *C. arvense* biomass from the first to third year (48.2 ± 40.2 to 1.9 ± 2.4), which coincided with two consecutive years of frequent tillage (Figure 3.8). Conversely, four sequences produced significantly more *C. arvense* biomass in the third year compared to the first (Figure 3.8). The SW-cereal forage (1.8 ± 2.3 to 43.9 ± 36.7), SW-lentil-pea (7.3 ± 6.8 to 24.8 ± 21.1), lentil-pea-hemp (24.6 ± 20.9 to 93.0 ± 79.9), and CCM-SW (5.6 ± 5.4 to 23.8 ± 20.3) sequences all had greater average *C. arvense* biomass in the final year compared to the first year of the study (Figure 3.8). All crop sequences with greater *C. arvense* biomass in the final year had three consecutive years of annual crops. In addition, the SW-cereal forage, SW-lentil-pea, and lentil-pea-hemp sequences also had crop failures in millet and sweet clover. Without successful millet and sweet clover crops, both sequences had almost 100 less days of competition with *C. arvense* than originally planned (Table 3.3).

Table 3.7. *Cirsium arvense* aggregated biomass (g per m^2) at Ft. Ellis Research Farm (FE; Bozeman, MT, USA) as effected by crop sequence, year of crop phase (Y1, Y2, etc.), tillage method, and their interactions as determined by an analysis of variance (ANOVA) for a linear mixed effects model.

Fixed Effects	Numerator DF	Denominator DF	F statistic	p-value
Crop sequence	7	28	1.6	0.16
Year of crop sequence	2	62	10.4	**< 0.001**
Tillage method	1	4	0.1	0.78
Crop sequence: year	14	62	8.6	**< 0.001**
Crop sequence: tillage	7	28	1.0	0.46
Tillage: year	2	62	0.4	0.64
Crop sequence: tillage: year	14	62	0.6	0.82

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

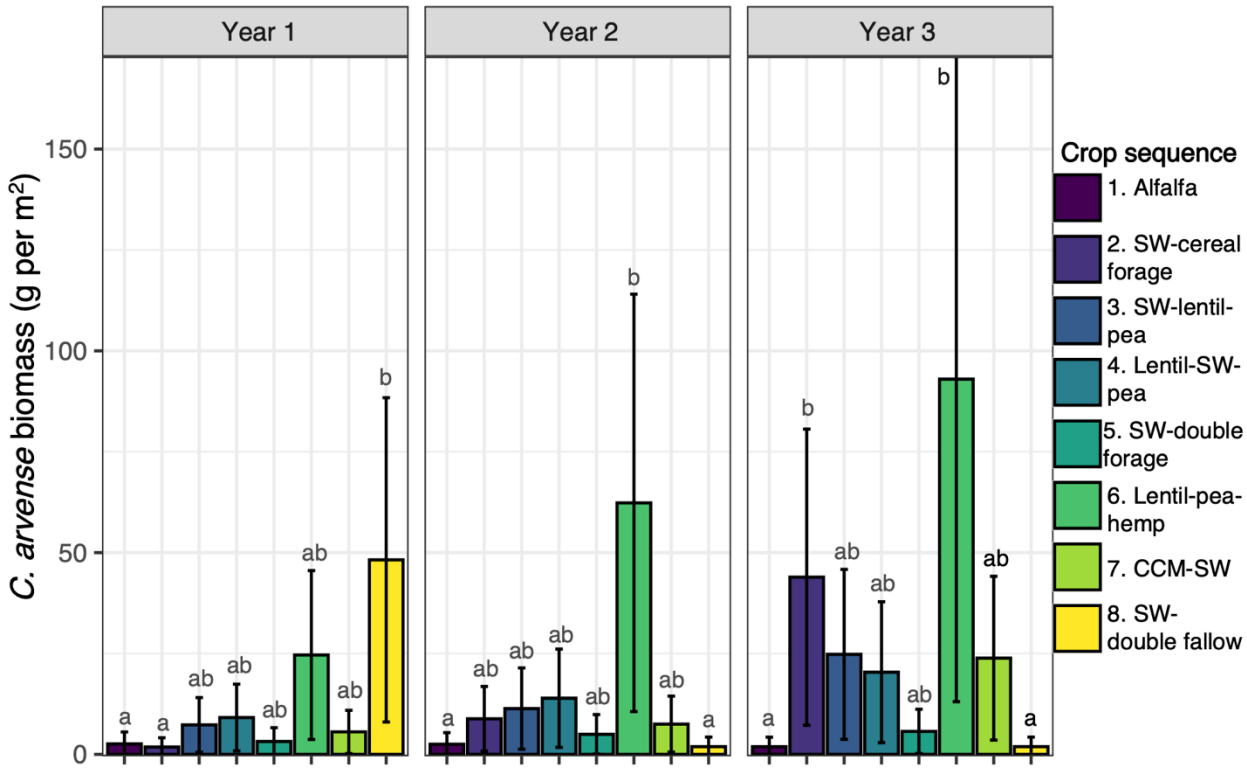


Figure 3.7. *Cirsium arvense* estimated mean biomass (g per m^2) within the eight crop sequences at Ft. Ellis Research Farm (FE; Bozeman, MT, USA) across years of the study. Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in mean biomass among crop sequences within year.

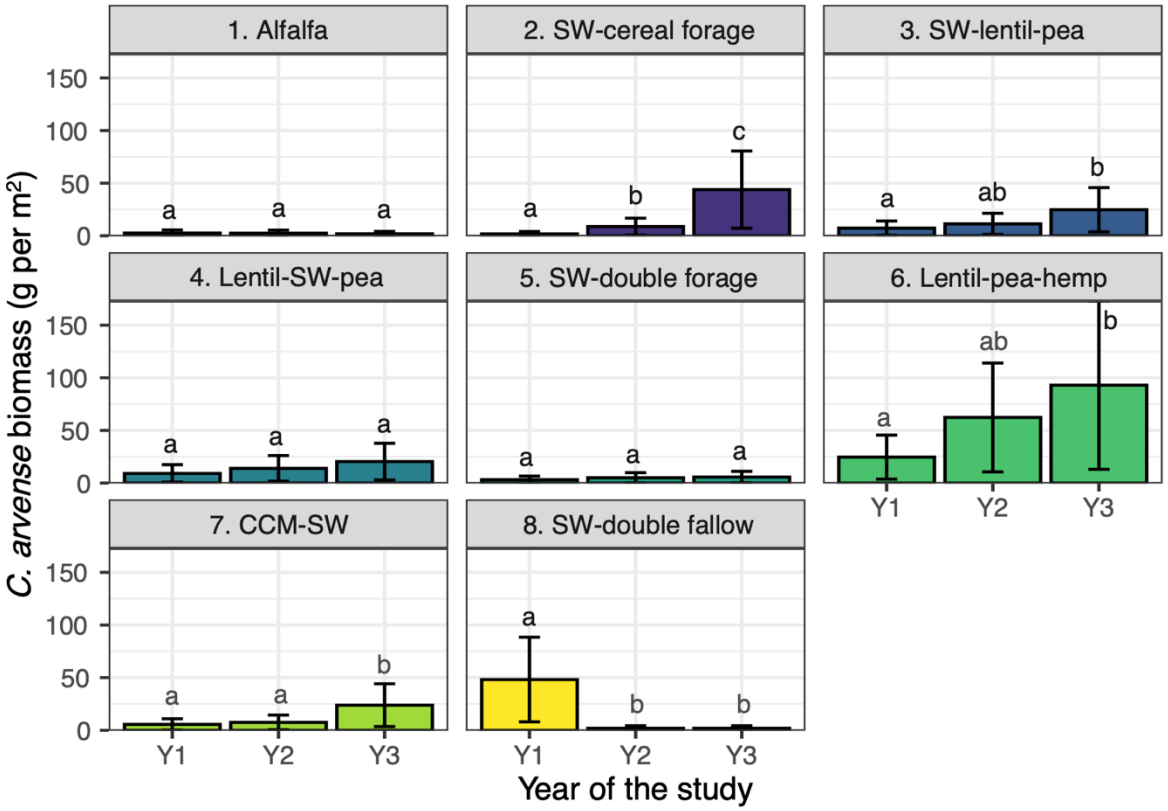


Figure 3.8. *Cirsium arvense* estimated mean biomass (g per m^2) in the eight crop sequences at Ft. Ellis Research Farm (FE; Bozeman, MT, USA) across years of the study. Standard errors for estimate means are given. Letters indicate post-hoc differences ($P < 0.05$) in mean *C. arvense* biomass within a crop sequence between years.

Discussion

In this study, *C. arvense* density and biomass increased in most crop sequences that were characterized by multiple years of annual crops. In contrast, *C. arvense* populations were low in sequences consisting of either perennial alfalfa or wheat followed by two consecutive years of tilled fallow. These results do not support the use of more diverse annual crop sequences as major components of IWM management plans for perennial *C. arvense* in dryland organic systems. Rather, our results support the hypothesis that a longer-growing, competitive perennial crop like alfalfa or substantial mechanical cultivation during fallow can suppress *C. arvense*

growth and spread. Integrating a temporary perennial forage into an annual grain sequence as part of a longer crop rotation or using intensive tillage in infested areas of a field can suppress *C. arvense* populations in organic systems. In some areas of the NGP and other dryland areas where soil erosion is a concern, the potential to substitute mechanical cultivation with planting of a competitive perennial forage may be a more sustainable solution for organic grain farmers.

Alfalfa is a good competitor with perennial weeds (Favrelière et al. 2020; Grosse et al. 2021). The competitive nature of alfalfa with *C. arvense* is two-fold: (1) competition for light through quick springtime growth; and (2) direct competition for water and soil nutrients through a far-reaching root system (Favrelière et al. 2020). Previous research indicated alfalfa grew more quickly than *C. arvense* in the early spring, leading to a closed canopy and preventing *C. arvense* from accessing direct sunlight (Hodgson 1958). As a shade-intolerant species, early canopy closure from alfalfa can stunt *C. arvense* growth and hinder its photosynthetic ability, thereby reducing its overall root and flower formation (Tiley 2010).

Root system length and size influence the overall competitive ability of a species (Casper & Jackson 1997). In a review of root distribution of agricultural crops, Fan et al. (2016) reported that alfalfa had the deepest potential rooting depth (1.8 m) of all crops. However, the maximum sampling depth of all alfalfa studies reviewed was only 1.8 m, suggesting that the perennial root system of alfalfa may be more extensive if environmental conditions allow. *Cirsium arvense* has a similarly deep and extensive root system, with the majority of roots existing in the top 30 cm of soil but few larger roots extending as far as 2 m (Donald 1994; Haderlie et al. 1987). A crucial component in the success of alfalfa with *C. arvense* may be the added disturbance in the form of a cutting or mowing event (Hodgson 1958). *Cirsium arvense* populations have reduced in

response to two or more cutting or mowing events (Bourdôt et al. 2016; Lukashyk et al. 2008). The added competitive pressure from established alfalfa may be enough to suppress *C. arvense* growth. Unlike perennial crops like alfalfa, most annual crops available in the NGP do not compete as well with perennial weeds like *C. arvense* (Entz et al. 2002; Favrelière et al. 2020). Annual root systems are less extensive and shallower than perennials, allowing such crops to be more susceptible to competition from established perennial weeds in early life stages (Schonebeck 2011).

Sweet clover as a crop and subsequent green manure suppressed perennial weeds in previous studies conducted in the semi-arid Canadian prairies, likely due to its extensive root system (Blackshaw et al. 2001; Moyer et al. 2007). Moyer et al. (2007) reported that available soil moisture was reduced after a sweet clover cover crop compared to a tilled fallow, but wheat yields following sweet clover were not diminished. In our study, biennial sweet clover was replaced with leguminous, annual field pea. In the current study, efforts to establish biennial sweet clover were unsuccessful because of severe defoliation from sweet clover weevil. Field pea was planted as a replacement crop for sweet clover which resulted in high *C. arvense* ramet densities at the end of the three-year study. Field pea, often integrated into semi-arid organic cropping systems for soil nitrogen contribution, is a non-competitive crop, especially with perennials like *C. arvense* (Annicchiarico & Filippi 2008; McKay et al. 2003). A successful sweet clover crop may have improved the overall competitiveness of these three sequences.

Interestingly, tillage method only appears to have effected *C. arvense* populations in its interaction with the factors of crop sequence and year, and only at the Ft. Ellis study site. Weed density and biomass is often greater in organic reduced tillage systems when compared to

organic fields under a standard tillage system (Hofmeijer et al. 2019; Larson et al. 2021; Zikeli & Gruber 2017). However, the relationship between crop choice and tillage system is still not fully understood, and many studies evaluating the importance of these two agronomic practices on weed populations have had mixed results (Demjanová et al. 2009; Grosse et al. 2021; Santín-Montanyá et al. 2013; Shrestha et al. 2002). A long-term study by Armengot et al. (2015) found organic reduced tillage plots to have 2.3 times greater weed abundance compared to standard tillage plots in the same crop sequence. In this experiment, however, we found no differences in *C. arvense* density or biomass between reduced or standard tillage plots within the same crop sequence. Livestock grazing was an important component of the reduced tillage system in this study. Livestock-integrated systems in the NGP have proven benefits in tillage reduction, improved soil quality and nutrient cycling, and expanded economic capabilities, but have not always successfully managed weed populations (Hatfield et al. 2007; Larson et al. 2021; McKenzie et al. 2016; Sainju et al. 2010). Our finding could have important implications for organic producers in the NGP, where reduced tillage tactics, including livestock grazing, are not widely implemented (OAEC 2013).

In our study, we saw that tillage system alone had no impact on perennial weed density and biomass. Rather, crop sequence, year, or their interaction explained greater variation in *C. arvense* populations. To improve integrated weed management, it is often suggested that tillage is combined with diversified and competitive crop sequences. We have demonstrated that, regardless of tillage, multi-year annual crop sequences do not successfully compete with new and established perennial weed populations as well as perennial forage or intensive tilled fallow. It is likely that non-competitive, shallow-rooted annual crops, such as forage peas and lentils, coupled

with low to moderate tillage intensity, was not suppressive to *C. arvense* and is not recommended for producers trying to manage this rhizomatous weed.

Conclusion

In this research, we demonstrated that perennial alfalfa preceded by a forage barley crop successfully suppressed *C. arvense* growth over a three-year period in dryland, organic grain systems. Wheat followed by two years of frequently tilled fallow (e.g., seven to eight tillage events per season) also maintained low *C. arvense* populations over the same period. Six other crop sequences, which were primarily composed of broadleaf annual crops, allowed for increases in *C. arvense* populations over time at one or both study locations. Failures in millet and sweet clover crop reduced the potential competitive impact of four treatments by reducing the total days of direct crop competition with *C. arvense* in the field. In the absence of a millet crop, *C. arvense* in the SW-cereal forage crop sequence went without any direct crop competition after the harvesting or grazing of triticale in midsummer, translating to 60 fewer days of plant competition. Without sweet clover establishment, a much less competitive field pea was seeded in three treatments and again shortened total days of crop competition with *C. arvense*. We found no impact of tillage system on *C. arvense* populations. The choice of crop sequence or yearly growing conditions had a greater impact on *C. arvense* populations than choice of a reduced or standard tillage system.

This study aimed to provide a substantive evaluation of multiple crop sequences and two tillage systems available to organic farmers in the dryland NGP. A broad span of annual crop sequences did not successfully suppress *C. arvense*. The application of a livestock-integrated or a traditional conventional tillage system did not affect *C. arvense* when compared to the impact of

crop choice or year. *Cirsium arvense* was not a particular strong competitor with an alfalfa crop. Our results suggest that organic farmers incorporate perennial forage or temporary perennial pasture into annual crop sequences to sustainably suppress perennial *C. arvense*. Our research also shows that the use of reduced tillage in an organic annual cropping system did not alone cause increases in *C. arvense* density or biomass. Longer-term studies (e.g., over four years) on *C. arvense* management using different perennial forage crops or a successful biennial sweet clover crop integrated into an annual grain sequence is recommended to complement our study.

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CHAPTER FOUR

SUMMARY OF FINDINGS AND FUTURE RESEARCH

This research examined the use of integrated weed management to reduce perennial rhizomatous weeds in organic small grain cropping systems in Montana. The objective was to compare different combinations of cultural and mechanical controls to determine which, if any, treatments had singular or additive effects on both *C. arvensis* and *C. arvense* populations in organic cropping systems. Specifically, we hypothesized that 1) perennial crops would suppress *C. arvensis* and *C. arvense* more successfully than annual crops and 2) intensive tillage systems would suppress *C. arvensis* and *C. arvense* better than reduced tillage systems.

Cultural weed management focuses on having high crop competition and diverse crops and crop sequences to alter weed selection pressure. In this study, crops in the sequences included had a range of different traits, including monocot and dicot traits and annual, biennial, and perennial life cycles. We expected that perennial alfalfa would suppress *C. arvense* and *C. arvensis* given its equally deep and extensive root system and early springtime growth and canopy cover (Favrelière et al. 2020; Grosse et al. 2021). In addition to direct competition for resources like soil moisture and nitrogen, alfalfa could quickly cover both weeds and limit their exposure to direct sunlight (Hodgson 1958; Stahler 1948; Tiley 2010). We also hypothesized that shallower-rooted annual crops such as field pea and lentil would allow for increases in both *C. arvense* and *C. arvensis* populations due to low level of competition and less days of direct plant competition (Meiss et al. 2010; Tautges et al. 2017).

Mechanical management of weeds was also assessed and spanned a gradient from absent in the case of perennial alfalfa to intensive and frequent soil inversion in two-year tilled-fallow

treatments. In the middle of the gradient was the integration of minimal to moderate tillage or livestock grazing with monocot and dicot annual crops. Within crop sequences, there were both standard frequencies of tillage and reduced tillage which incorporated sheep grazing to minimize mechanical disturbance of soil. Given the regrowth of perennial rhizomatous weeds after root disturbance, we expected that reduced tillage systems, especially those reliant on sheep grazing and non-inversion or infrequent tillage, would produce greater populations of *C. arvense* and *C. arvensis* (Carr et al. 2013; Larson et a. 2021; Rusu et al. 2013). Conversely, we hypothesized that intensive or more frequent tillage (e.g., six to eight tillage events a season) would suppress populations of *C. arvense* and *C. arvensis* over time given it would adequately disrupt the extension perennial root systems of both plants (Arif et al. 2007; Hodgson 1958; Melander et al. 2012).

Summary of Findings

As seen in previous research, perennial alfalfa forage limited the growth of *C. arvense* and *C. arvensis* populations. Crop sequences which had greater frequency of both monocot and dicot annual crops, such as triticale, field pea, or lentil, increased in *C. arvense* and *C. arvensis* densities over three field seasons. In particular, treatments with consecutive years of annual crops saw the greatest increases in perennial weed density and biomass. These observations are potentially due to a lack of competitive characteristics in annual crops, such as smaller root systems, earlier termination dates, and subsequently less days of overall direct competition with perennial weeds. Holt and Orcutt (1991) demonstrated three perennial weeds (*Cyperus* spp. and *Sorghum halepense*) presented a greater number of growth variables (e.g., relative growth rate; height; light utilization) and was significantly correlated with greater overall competitiveness

when compared to an annual crop (*Gossypium hirsutum*). Annual leguminous, nitrogen-fixing crops, like field pea, may have actually improved growing conditions for *C. arvense* and *C. arvensis* by adding a stable source of available nitrogen to the soil (e.g., Jensen 1996). Overall, the three consecutive years of annual crops in organic systems was detrimental to weed suppression, whereas the integration of a three-year perennial forage was highly competitive with perennial *C. arvense* and *C. arvensis*.

We also found that tilled fallow treatments had low perennial weed density and biomass. Results with tillage became less successful, however, when different tillage systems were integrated into more diversified crop sequences. With *C. arvense*, for example, the interaction of crop sequence and year affected weed density and biomass, but tillage method alone did not. For *C. arvensis*, a tilled-fallow system was again successful in maintaining low weed densities, but minimal to moderate frequency tillage with more diverse crop sequences were not. Overall, this study indicates that the reality of integrated weed management for perennial weeds may be more limited in dryland organic grain systems than previously suggested. If perennial weed suppression is a primary goal for an organic grain operation, our results indicate that multiple years of perennial forage cover should be employed. While a tilled fallow system can suppress perennial weeds, adverse effects on soil structural quality were observed in our study on *C. arvensis* and is therefore not recommended as a sustainable, large-scale option in areas like the NGP where soil erosion is a major concern.

Future Directions

Weed species remain a top concern for organic producers worldwide (Bàrberi 2002; Gomiero et al. 2011; OAEC 2013). In grain producing areas of Montana, finding successful

management strategies for perennial rhizomatous weeds is imperative for organic farmers (Orloff et al. 2018). While understudied for use against *Cirsium arvense* and *Convolvulus arvensis*, the concept of integrated weed management as a method for weed suppression in organic systems has been widely proposed and accepted (Merfield 2019; Mohler et al. 2019). However, our studies highlighted the complex nature of using integrated weed management in controlling perennial rhizomatous weeds in dryland organic agricultural settings. Perennial alfalfa, maintained with either livestock grazing or cutting, and frequent tillage, using either a reduced or conventional method, managed perennial weed populations equally well. More highly integrated systems using diverse crops and varied forms and intensities of soil cultivation showed no success in suppressing either *C. arvensis* or *C. arvense*. It has been suggested that combinations of biological, cultural, and mechanical control tactics should achieve better weed control than a singular management tool (Buhler et al. 2002; Liebman et al. 1997; Swanton and Weise 1991). Our results, however, found very few tenable options for the management of perennial weeds in semi-arid organic systems. The best weed management outcomes in this experiment did not include integrating techniques, but rather relying almost solely on a competitive perennial crop or intensive mechanical cultivation.

Integrated weed management suggestions need to be refined for organic, small-grain farmers in semi-arid Montana. Adding appropriate cover crops to crop sequences in organic grain systems has been suggested, with respect to soil moisture concerns, to benefit system components like total grain production (Hansen et al. 2016; Smith et al. 2017). However, we demonstrated that increasing diversification of crop sequences with less competitive annual crops led to sharp increases in perennial weed populations over only a short period. Annual

crops, especially when spring-seeded, do not have suitable characteristics for competing with even small populations of established perennial weeds (Meiss et al. 2010; Schoof and Entz 2000; Stevenson and Johnston 1999). Annual forage crop harvest or grazing often occurs too late in a perennial weed's life cycle to be impactful (e.g., grazing occurring while weeds are flowering, instead of while they are bolting) (Bourdôt et al. 2016).

Future research could focus explicitly on the timing of mowing, grazing, and harvesting operations of common annual crops in Montana in conjunction with the physiological timeline of both *C. arvensis* and *C. arvense*. Such work would better address the shortcomings of crop diversification as an integrated weed management strategy and aid in determining what crop characteristics would be most beneficial to manage perennial weeds. In addition, this research area could benefit from future studies having extended timelines (e.g., longer rotations than four years). Many diversified organic producers in Montana utilize crop rotations as long as nine years, with five years allotted to cash crops and four years allotted to soil-building (e.g., planting of leguminous annual or perennial crops to increase soil fertility or manage weeds) (Bob Quinn, personal communication). While alfalfa is a strong suppressant of perennial weeds, it is unlikely to be as economically viable to farmers as growing a cash grain crop (Delbridge et al. 2011). Future research would benefit from addressing the agronomic and economic tradeoffs of integrating a temporary perennial crop into an annual crop-based system. Overall, we recommend continued inquiry into the integration of alfalfa or perennial pastureland for managing perennial weeds into annual organic grain systems, with a specific focus on extending or repeating crop rotations.

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APPENDICES

APPENDIX A

SUPPLEMENTAL INFORMATION FOR CHAPTER TWO

Table S2.1. Estimated marginal means of *C. arvensis* ramet density per m^2 among ten cropping system treatments within years of the study. Letters indicate significant post-hoc differences in mean ramet density within a year ($P < 0.05$).

	Year			
Cropping Treatment	2017	2018	2019	2020
Tillage 1	0.3 ± 0.3a	0.43 ± 0.43a	0.012 ± 0.012a	0.009 ± 0.009ab
Tillage 2	0.3 ± 0.3a	0.44 ± 0.44a	0.13 ± 0.13ab	0.1 ± 0.1abc
Wheat-CC + till	0.9 ± 0.9a	1.13 ± 1.13a	4.01 ± 4.01b	5.06 ± 5.06c
Wheat-CC	0.23 ± 0.23a	0.47 ± 0.47a	2.5 ± 2.5b	0.19 ± 0.19abc
Spring till	0.3 ± 0.3a	0.094 ± 0.094a	0.37 ± 0.37ab	0.69 ± 0.69abc
Rye-CC + till	0.79 ± 0.79a	1.14 ± 1.14a	2.83 ± 2.83b	2.83 ± 2.83bc
Reduced till	0.54 ± 0.54a	0.12 ± 0.12a	0.99 ± 0.99ab	1.65 ± 1.65bc
Grazing-CC	0.71 ± 0.71a	1.29 ± 1.29a	3.05 ± 3.05b	6.75 ± 6.75c
Conventional alfalfa	0.21 ± 0.21a	0.27 ± 0.27a	0.25 ± 0.25ab	0.038 ± 0.038ab
Organic alfalfa	0.63 ± 0.63a	0.62 ± 0.62a	0.09 ± 0.09 ab	0.36 ± 0.36abc

Table S2.2. Estimated marginal means of *C. arvensis* population growth rates for ten cropping system treatments within four transition periods (e.g., 2017 to 2018).

	Year			
Cropping Treatment	2017-2018	2018-2019	2019-2020	2017-2020
Tillage 1	0.34 ± 0.96	-3.54 ± 0.96	0.36 ± 0.96	-3.56 ± 0.96
Tillage 2	0.45 ± 0.96	-1.21 ± 0.96	-0.28 ± 0.96	-1.05 ± 0.96
Wheat-CC + till	0.19 ± 0.96	1.27 ± 0.96	0.23 ± 0.96	1.69 ± 0.96
Wheat-CC	0.70 ± 0.96	1.68 ± 0.96	-2.60 ± 0.96	-0.22 ± 0.96
Spring till	-1.17 ± 0.96	1.38 ± 0.96	0.63 ± 0.96	0.82 ± 0.96
Rye-CC + till	0.37 ± 0.96	0.91 ± 0.96	0.00 ± 0.96	1.28 ± 0.96
Reduced till	-1.64 ± 0.96	2.24 ± 0.96	0.52 ± 0.96	1.11 ± 0.96
Grazing-CC	0.59 ± 0.96	0.86 ± 0.96	0.80 ± 0.96	2.25 ± 0.96
Conventional alfalfa	0.27 ± 0.96	-0.09 ± 0.96	-1.86 ± 0.96	-1.69 ± 0.96
Organic alfalfa	0.00 ± 0.96	-1.95 ± 0.96	1.41 ± 0.96	-0.54 ± 0.96

Table S2.3. Wheat yield (T/ha) as effected by cropping system treatment as determined by analysis of variance (ANOVA) derived from underlying linear mixed effects models.

Predictor	Sum Sq.	Mean Sq.	NumDF	DenDF	F-value	Pr(>F)
Treatment	0.81	0.090	9	18	1.37	0.27

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

Table S2.4. Output of the analysis of variance (ANOVA) of soil large macroaggregate percentage, soil macroaggregate percentage, and soil microaggregate percentage as explained by cropping system treatment.

Soil %	Predictor	Sum Sq.	Mean Sq.	NumDF	DenDF	F-value	Pr(>F)
Large macro	Treatment	68.35	7.6	9	18	1.02	0.46
Macro	Treatment	204.92	22.77	9	18	1.01	0.47
Micro	Treatment	121.05	13.45	9	18	0.74	0.67

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

Table S2.5. Multiple soil chemical variable values as explained by cropping system treatment as determined by analysis of variance (ANOVA) from the individual, underlying mixed effects models.

Variable	Sum. Sq.	Mean Sq.	NumDF	DenDF	F-value	Pr(>F)
P (ppm)	844.80	93.90	9	18	2.50	0.05
K (ppm)	5541.40	615.70	9	18	6.30	0.005
Zn (ppm)	0.07	0.01	9	18	4.30	0.05
OM	0.20	0.02	9	18	1.40	0.4
pH	0.50	0.05	9	18	0.50	0.8
Salt1	0.03	0.00	9	18	1.70	0.2
Ca (ppm)	2608495.00	289833.00	9	18	1.00	0.5
Mg (ppm)	1379.00	153.20	9	18	0.60	0.8
Na (ppm)	94.50	10.50	9	18	1.00	0.4
CEC (meq/100g)	65.00	7.20	9	18	1.00	0.5
Total N (ppm)	181030.00	20114.00	9	18	1.20	0.4
Total N (%)	0.00	0.00	9	18	1.20	0.4
NH_4 (ppm)	0.50	0.05	9	18	1.20	0.4
S1 (lb)	2722.10	302.50	9	18	0.60	0.8

Note: ANOVA table using type III analysis of variance with Satterthwaite's method.

APPENDIX B

SUPPLEMENTARY MATERIALS FOR CHAPTER THREE

Table S3.1. Precipitation and temperature data for the study sites Ft. Ellis Farm (Bozeman, MT) and Central Agricultural Research Center (Moccasin, MT) from 2019 to 2022.

	Precipitation (mm)					Mean temperature (°C)				
	2019	2020	2021	2022	LTA[A]	2019	2020	2021	2022	LTA
					Bozeman					
Sep-Mar[B]	N/A	287	173	78	218	N/A	1.4	-2.7	-1.8	2.5
Apr	N/A	30	44	78	59	N/A	5.2	5.8	3.2	6.6
May	N/A	45	81	148	86	N/A	11.7	10.2	9.4	11.1
June	N/A	90	28	80	81	N/A	15.4	19.5	15.7	15.4
July	N/A	16	26	22	38	N/A	19.0	22.8	21.5	18.7
Aug	N/A	23	84	30	38	N/A	20.1	18.5	22.1	19.1
Growing season[C]	N/A	204	264	358	301	N/A	14.3	15.4	14.4	14.2
					Moccasin					
Sep-Mar	161	154	82	85	130	-0.5	1.6	2.4	2.2	1.6
Apr	47	20	26	47	36	5.4	2.6	4.6	1.1	6.2
May	94	68	68	35	70	8.1	10.4	9.7	8.5	11.2
June	69	96	15	41	70	14.3	15.0	18.0	14	15.7
July	49	24	23	73	48	17.9	18.2	22.7	20.6	20.2
Aug	49	9	68	27	50	17.9	20.5	18.0	20.9	19.9
Growing season	308	217	200	223	275	12.7	13.4	14.6	13.0	14.6

[A] Long-term average, 1980-2010 from the Western Regional Climate Center, Desert Research Institute, Reno, NV

[B] Over-winter preceding the growing season (1 September to 31 March)

[C] 1 April to 31 August

Appendix S3.2. Model fitting for *Cirsium arvense* density.

Cirsium arvense density was collected as ramet count data from six randomized 0.5 m^2 sampling frames within unique plots or subplots at both study sites (Central Agricultural Research Center [CARC] and Ft. Ellis Research Farm [FE]). Frame data was first aggregated within plots (CARC) or subplots (FE) and scaled to 1 m^2 to avoid pseudo-replication and achieve appropriate degrees of freedom. Raw aggregate data visualizations were assessed to consider general trends across both sites (Figures S3.1-S3.4). Initial reviews of tillage at both sites showed relatively similar means for standard ("st") and reduced tillage ("rt") for *C. arvense* density within crop sequences (Figures S3.1 and S3.2). When evaluating crop sequences over time, certain sequences ("CS1" [Alfalfa] and "CS8" [Double fallow]) to had low mean *C. arvense* densities across both sites (Figure S3.3 and S3.4). Other crop sequences ("CS3" [Wheat-lentil-pea] or "CS7" [Wheat-cover crop mix]) appeared to demonstrate year-to-year increases in *C. arvense* density at both study sites. Other crop sequences ("CS5" [Wheat-double forage] and "CS6" [Lentil-pea-hemp]) had much more variable *C. arvense* density.

Figure S3.2.1. Boxplot visualizations of differences in *C. arvense* density per m^2 at the Ft. Ellis Research Farm by tillage system (reduced till "RT" and standard till "ST") and crop sequence ("CS") averaged across the first three years of the study.

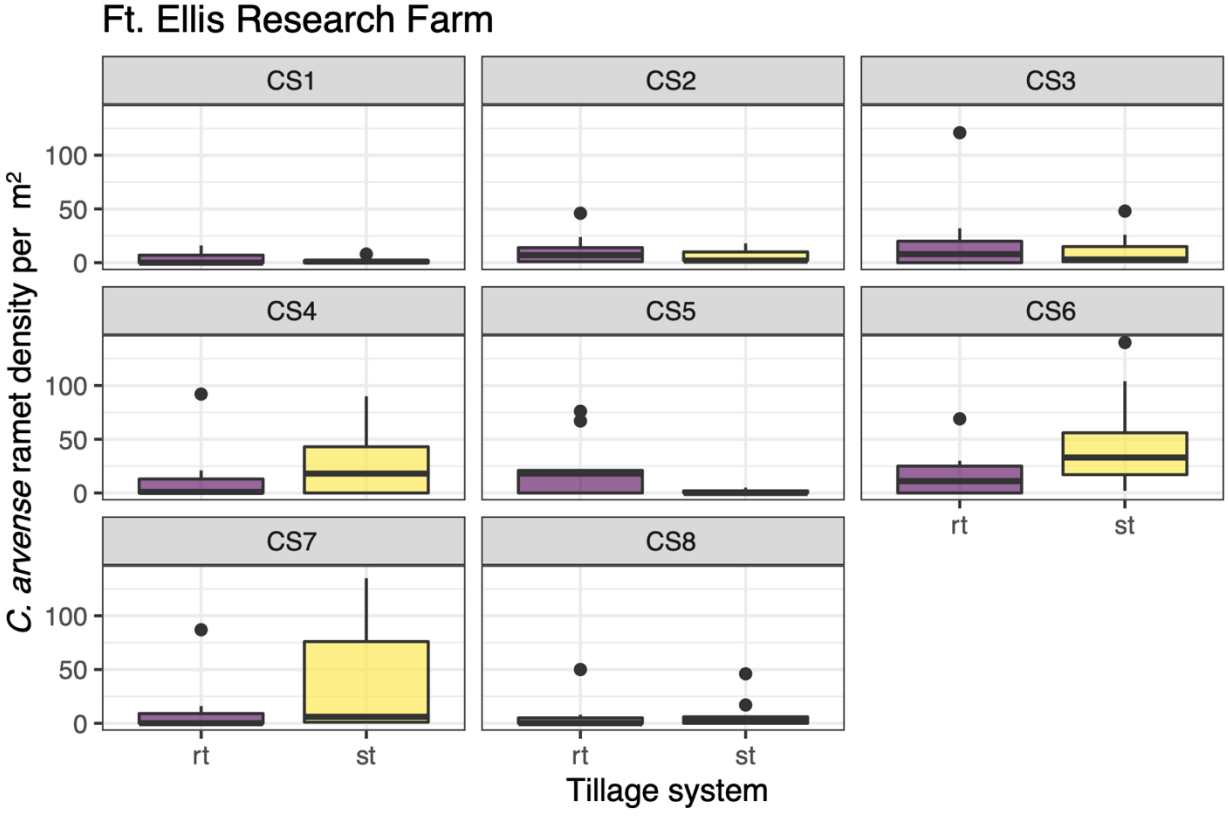


Figure S3.2.2. Boxplot visualizations of differences in *C. arvense* density per m^2 at Central Agricultural Research Center (Moccasin, MT, USA) by tillage system (reduced till "RT" and standard till "ST") and crop sequence ("CS") averaged across the first three years of the study.

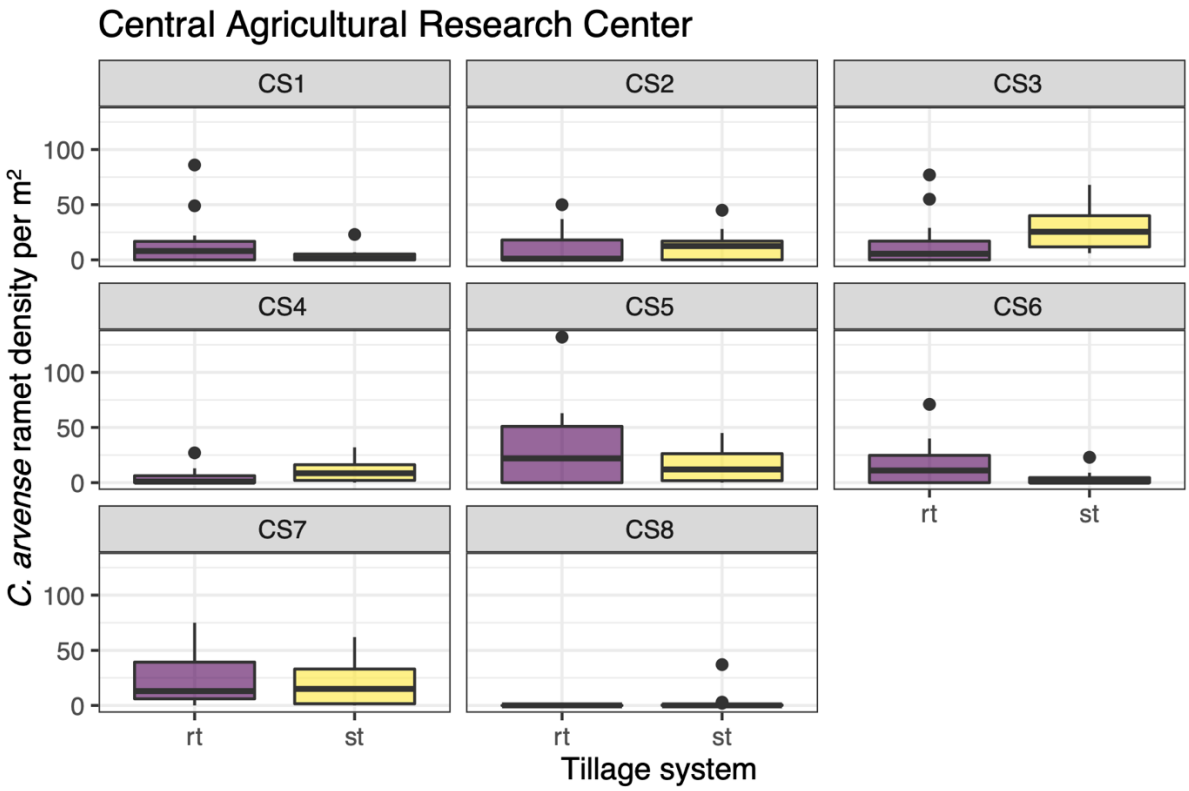


Figure S3.2.3. Boxplot visualizations of differences in *C. arvense* density per m^2 at Ft. Ellis Research Farm (Bozeman, MT, USA) by crop sequence ("S") across the first three years of the study (Y1, Y2, Y3).

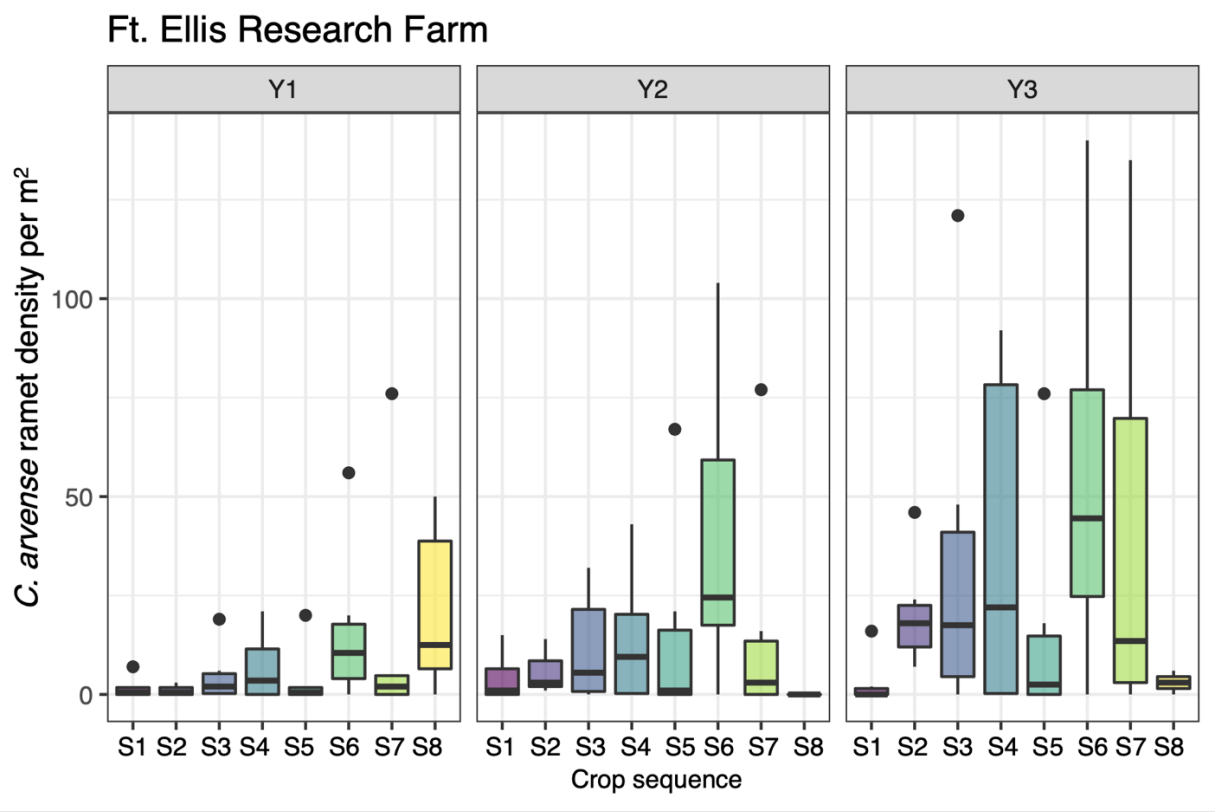
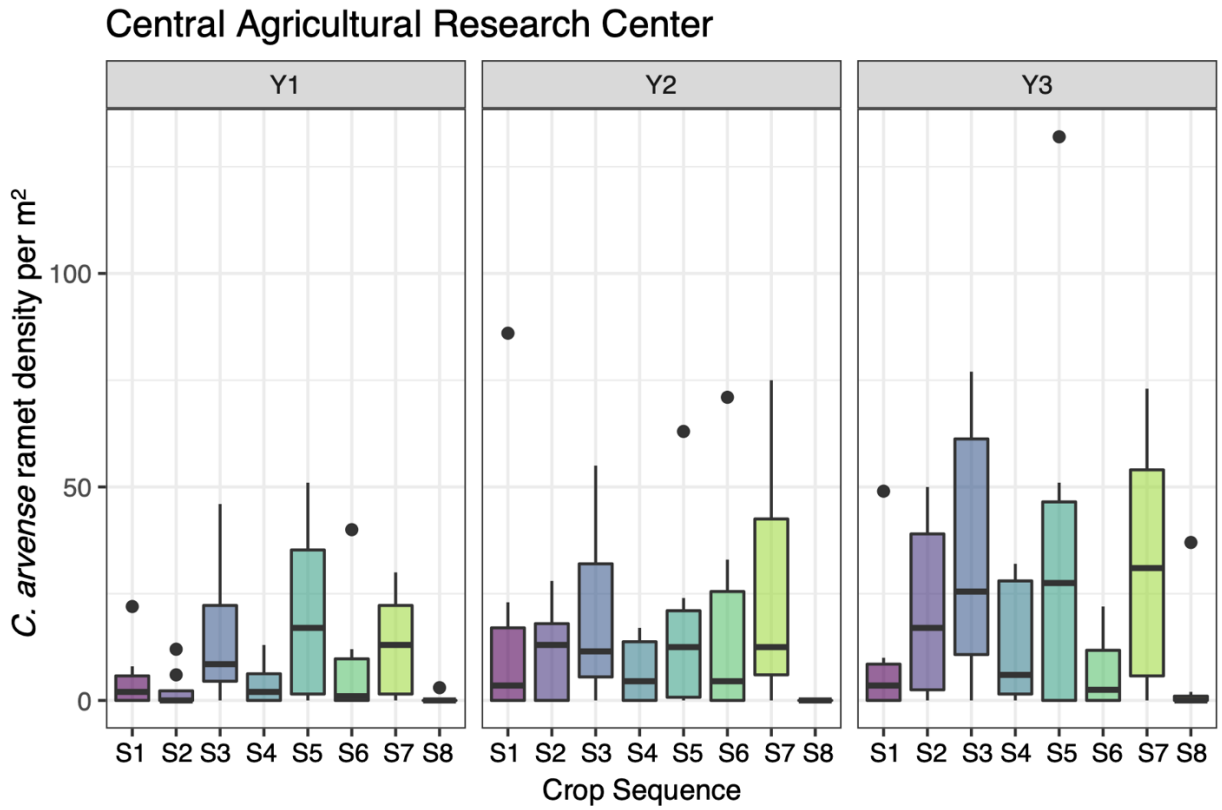


Figure S3.2.4. Boxplot visualizations of differences in *C. arvense* density per m^2 at Central Agricultural Research Center (Moccasin, MT, USA) by crop sequence ("S") across the first three years of the study (Y1, Y2, Y3).



Even though data was aggregated from frames to create an average *C. arvense* density value for each plot or subplot, there was still a high number of true zeroes (e.g., *C. arvense* was absent from frames or plots) and a large range of density values (e.g., plots with zero counts of *C. arvense* and plots with over 200 counts of *C. arvense*) in the aggregated data sets (Figures S3.5 and S3.6). As a creeping, rhizomatous perennial, *C. arvense* has naturally patchy establishment (e.g., high abundance at a particular plot or block but absent in the neighboring plot or block). This patchy distribution appeared to be true at our two study sites. The FE site had well-established populations of *C. arvense* prior to the start of the study. It is likely the CARC site also had the majority of *C. arvense* populations establish from neighboring *C. arvense* populations at the start of the study.

Figure S3.2.5. Histogram of *C. arvense* density count values at Central Agricultural Research Center (CARC; Moccasin, MT, USA) across years of the study.

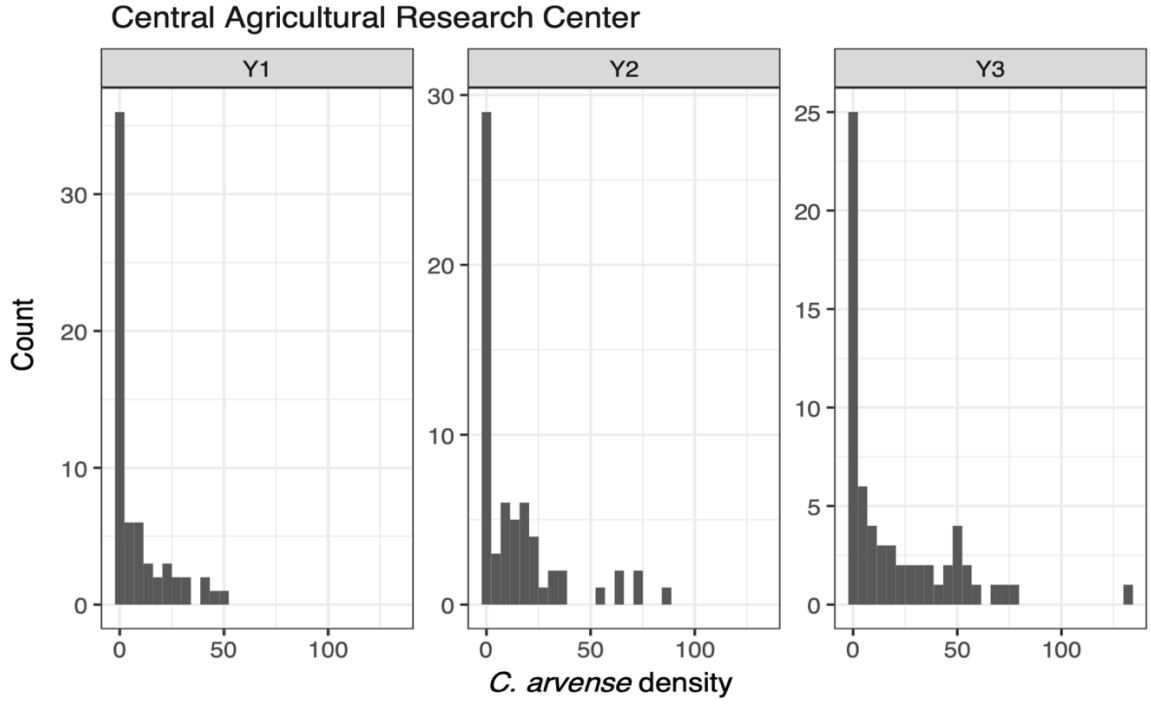
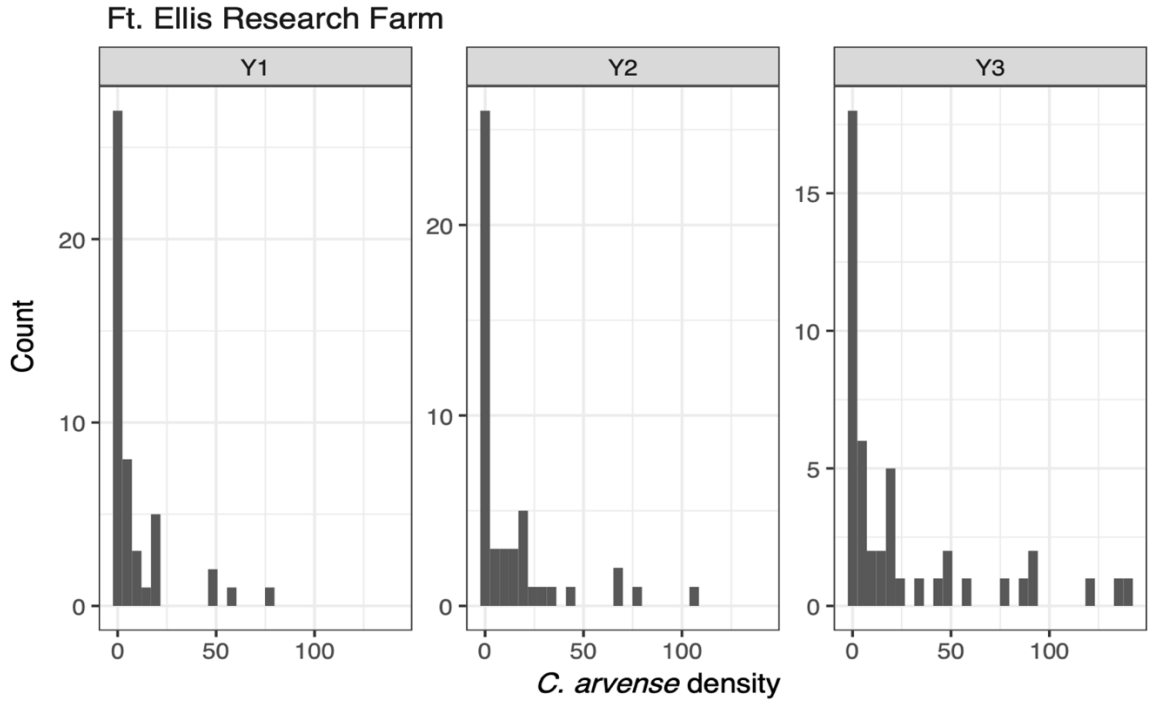


Figure S3.2 .6. Histogram of *C. arvense* density count data at Ft. Ellis Research Farm (FE; Bozeman, MT, USA) across years of the study.



We started by fitting a generalized linear mixed effects model (GLMER) with a Poisson distribution for our aggregated count data. Because aggregated data is an average across frames and therefore not necessarily integers, we had to round aggregated density values to the nearest whole number to utilize a Poisson distribution. Year of the study (e.g., Y1, Y2, Y3), tillage system (e.g., RT or ST), and crop sequence (e.g., CS1, CS2, etc.) and their interactions were used as fixed effects in the model, and unique plot identification and block were used as random effects (`glmer` [family = Poisson] in R package 'lme4'; Bates 2015). For CARC, most variance was accounted for by unique plot identification (3.1), whereas block accounted for much less variation (0.7). Conversely at FE, higher variance was seen in the blocking effect (3.2) when compared to unique subplots (1.0). A Type-III Anova (`Anova` function from R package 'car'; Fox & Weisberg 2019) for the CARC model found year, the interaction of year and crop sequence, and the three-way interaction of year, crop sequence, and tillage to be significant. For the FE model, crop sequence, the interaction of year and crop sequence, the interaction of tillage and crop sequence, and the three-way interaction of year, crop sequence, and tillage impacted *C. arvense* density.

Though recommended for count data, the GLMER model with a Poisson distribution for CARC exhibited significant overdispersion ($P < 0.00001$). Additionally, the fitted residuals plot and normal QQ plots showed that the data did not meet the assumption of normality nor the assumption of homoscedasticity. The GLMER model with a Poisson distribution for FE did not demonstrate overdispersion, but had very large standard errors, likely due to the high level of variance in the random effects. It was therefore deemed necessary to explore other model types to see if there were distributions better fit for our data.

A generalized linear mixed effects model with a negative binomial distribution were then explored for *C. arvense* density data (`glmer.nb` in R-package 'lme4'; Bates et al. 2015). Negative binomial distributions are recommended specifically when overdispersion occurs within discrete count data (Stoklosa et al. 2022). It is likely the high number of true zeroes in the data is leading to the overdispersion. Overdispersion is a concern because it can lead to overestimations of model parameters and therefore incorrect interpretation of results. Due to the control on overdispersion, negative binomial distributions can sometimes be more conservative estimates of model parameters. Additionally, a negative binomial model can allow for the aggregated average to be run without rounding values to whole integers, unlike a Poisson.

Full interactive models were first fit for both CARC and FE. Again, year of the study (e.g., Y1, Y2, Y3), tillage system (e.g., RT or ST), and crop sequence (e.g., CS1, CS2, etc.) and their interactions were used as fixed effects and unique plot identification and block were used as random effects (`glmer.nb` in R-package 'lme4'). The GLMER.NB model corrected overdispersion for CARC ($P = 0.99$). Variance remained unchanged from the Poisson model (unique plots = 2.0; blocks = 0.8). The full interactive model for CARC found year and the interaction of year and crop sequence to effect *C. arvense* density. The full interactive negative binomial model for FE aggregated data was slightly under-dispersed (0.94). Variance remained unchanged from the Poisson model with block accounting for 3.2 and unique plot identification accounting for 1.0. In the negative binomial model, the same variables and interactions appeared

significant as in the Poisson. Because of the significant reduction in overdispersion in the CARC model and no statistically perceivable change in outcomes of the FE model, the generalized linear mixed effects model with a negative binomial distribution was chosen for both sites.