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Canada thistle management

Integrated management of Canada thistle (*Cirsium arvense*) in the Great Plains and Intermountain West using a biocontrol agent (*Puccinia suaveolens)*

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Abstract

Canada thistle [*Cirsium arvense* (L.) Scop.] is an invasive perennial plant that threatens agricultural landscapes and natural ecosystems worldwide. The extensive regenerative root system of *C. arvense* complicates control efforts, with current strategies having limited success. *Puccinia suaveolens* (Pers.) Rostr (syn. *Puccinia punctiformis* (F. Strauss) Rohl), an obligate biotrophic rust fungus, has shown potential as a biological control agent by systemically infecting the root system, reducing root mass and shoot growth, and limiting vegetative regeneration; however, its efficacy when integrated with other control methods remains unclear. We conducted experiments from 2020 to 2022 at two sites in Colorado and Utah to evaluate *P. suaveolens* efficacy when applied alone and in combination with mowing, tillage, and herbicide. Treatments were applied in Fall (2020 and 2021), with monitoring of thistle stem density, vegetative cover, as well as *P. suaveolens* incidence before and after treatments through 2022. While *P. suaveolens* alone contributed to a decrease in thistle density, it was far less effective compared to herbicide treatments, and its impact when integrated with mowing or tillage was inconsistent. Herbicide application (alone and when combined with *P. suaveolens*) generated the greatest immediate reduction in thistle stem density and vegetative cover, although it resulted in the greatest amount of bare ground exposure. Grass coverage present within plots varied significantly between treatments, ranging from 0-75%, with the highest percentage observed in herbicide treatments in both years. Forb cover remained below 30% across treatments and years. Although *P. suaveolens* can contribute to *C. arvense* suppression, additional research is needed to remove barriers to its successful establishment, systemic infection and spread within populations, which could improve upon its efficacy, and optimization when integrated with other control strategies.

Keywords: aminopyralid, biological control, chlorsulfuron, integrated weed management, rust pathogen, thistle, stem count m^2

Introduction

Canada thistle [*Cirsium arvense* (L.) Scop.] (Asteraceae) is a pervasive perennial weed found throughout temperate regions globally. In the Western United States (U.S.), *C. arvense* ranks as one of the highest among the most commonly occurring noxious weeds, posing significant threats to both managed and natural landscapes (Bodo Slotta et al. 2010; Moore et al. 1975; Nuzzo 1997). In agricultural systems, *C. arvense* competes for light, nutrients, and water, leading to reduced crop yield and quality, generating significant economic losses for producers (Jacobs et al. 2006; Moyer et al. 1991; O'Sullivan 1982). In natural systems, it similarly competes with and displaces native plant species (Jacobs 2006). *Cirsium arvense* is commonly found in disturbed areas, including roadsides, streambanks, ditches, clear cuts, forest openings, and wet or wet-mesic grasslands and rangelands, as part of the initial post-disturbance community (Morishita 1999; Nuzzi 1997). It is prevalent in nearly every upland herbaceous community within its range, particularly prairie communities and riparian habitats (Nuzzo 1997).

Cirsium arvense survives and spreads through two reproductive strategies: sexual reproduction via seeds and clonal vegetative growth. Seeds are small and light, with highly variable germination success (Hodgson 1964; Moore 1975; Nuzzo 1997); however, seeds are important for range expansion as shown by the genetic diversity of North American populations (Bodo Slotta et al. 2010). Once established, the plant develops a creeping root system, up to 2-3m belowground, with adventitious root buds resulting in clonal vegetative growth that enables rapid propagation and spread (Donald 1994; Lalonde and Roitberg 1994). The adventitious buds develop into new rosettes and lateral roots that continue to grow throughout spring, summer, and into fall. As temperatures decrease in fall, the aboveground vegetation dies off, and the roots overwinter; in the spring, root growth resumes and new shoots emerge (Lalonde and Roitberg 1994; Tiley 2010). Fragments of roots as small as 1cm are capable of regenerating and forming new colonies (Nadeau and Vanden Born 1989; Thomsen et al. 2015). The complex and extensive root system of *C. arvense* allows for propagation, spread, and recovery making it particularly problematic and challenging for management (Nadeau and Vanden Born 1989; Tiley 2010).

Several management tactics are regularly utilized in efforts to control *C. arvense*. Chemical control is common and effective, generally providing rapid results. Mowing can also be an effective control method by reducing photosynthetic capacity aboveground and depleting root reserves used for regrowth, leading to a reduction of new shoots the following season (Bourdôt et al. 2011; Graglia et al. 2006). Similarly, cultivation and tillage fragment the root system and force the plant to use root reserves for recovery, however this may also promote new shoot development and further spread of *C. arvense* (Graglia et al. 2006, Thomsen et al. 2015). Both tillage and mowing are most effective when integrated into a weed management program to control established populations. While most current management of *C. arvense* relies on either herbicides, mowing, or cultivation, these tactics can be costly, labor-intensive, and often not ideal for environmentally sensitive areas (e.g., riparian zones) (Bourdôt et al. 2011; Graglia et al. 2006; Peterson et al. 2020; Thomsen et al. 2015). In contrast, biological control tactics are often more suitable for managing weeds in natural areas, as they can be self-perpetuating, and more economical in landscape-wide suppression of target species (Cripps et al. 2011; Guske et al. 2004; Peterson et al. 2020; Sciegienka et al. 2011).

Effective biological control has long been sought for *C. arvense*. *Puccinia suaveolens* (Pers.) Rostr (syn. *Puccinia punctiformis* (F. Strauss) Rohl), an obligate biotrophic rust fungus, was first proposed as a control method for *C. arvense* in 1893 in North America (Wilson 1969). *Puccinia suaveolens* can be naturally found on *C. arvense* plants throughout its growing region, commonly co-introduced in the invasive range and causing periodic outbreaks of disease (Berner et al. 2013; French and Lightfield 1990). Highly host specific to *C. arvense*, *P. suaveolens* has only been reported on two other thistles native to Eurasia; *Silybum marianum* (L.) Gaertner in 2002 under greenhouse conditions and *C. setosum* (Willd.) M. Bieb. in 2023 under field conditions in China (Berner et al. 2002; Liang et al. 2024). The potential for *P. suaveolens* to be utilized more broadly as a biocontrol for *C. arvense* will increase with continued research (Bean et al. 2024; Berner et al. 2015a; Berner et al. 2015b; Cripps et al. 2014; Thomas et al. 1994).

The lifecycle of *P. suaveolens* can be divided in two stages: the vegetative mycelium within the root system and the spore-producing aboveground systemic and local infections. It is thought that *P. suaveolens* remains latent within the root system until abiotic or biotic conditions are adequate to produce spore-bearing systemically infected stems (Mendgen and Hahn 2002). *Puccinia suaveolens* infection reduces *C. arvense* belowground biomass as root resources are parasitized by mycelia and allocated to plant defense compounds instead of growth (Chichinsky et al. 2023; Clark et al. 2020). This reduces the aboveground shoots and competitive ability of *C. arvense* (Chichinsky et al. 2023). Systemically infected stems typically do not flower, can die off early in the season and may help to further reduce root resources of infected *C. arvense* colonies

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(Van den Ende et al. 1987; Chichinsky et al. 2023). The rate and distance of spread of *P. suaveolens* caused by underground mycelia or aboveground spores remains unknown.

Puccinia suaveolens produces five spore types that develop consecutively beginning with emergence of systemically infected shoots in early spring that are deformed, chlorotic, strongly floral scented, and covered in yellow-orange pycnia pustules (Buller 1950; Menzies 1953; Petersen 1974). After outcrossing, pycniospores give rise to chain-like formations of the dark orange-brown aeciospores giving the systemically infected stems a characteristic rusty appearance (Berner et al. 2013; Connick and French et al. 1991). Production of urediniospores is indicated by the darkening of the spores (Buller 1950; Peterson 1974). Urediniospores and aeciospores are morphologically indistinguishable as single celled spores but only urediniospores produce localized infections on neighboring plants throughout summer (Kirk et al. 2001, Menzies 1953; Peterson 1974). Localized infections produce pustules on the leaves but the shoots do not have the same growth abnormalities associated with systemic infection, and will still appear relatively normal (Baka and Lösel 1992; Thomas et al. 1994). Localized infections can produce two-celled teliospores on leaves that senesce heading into fall, then the spores will either blow off and overwinter in the soil or germinate on new rosettes to initiate new vegetative infection in the roots or systemic infection aboveground. (Alexopoulos et al. 1996; Berner et al. 2015b; Menzies 1953). Optimal teliospore germination occurs when temperatures are between 16-20C (Berner et al. 2013; French and Lightfield 1990) with optimal dew periods of 2-3 hours (Morin et al. 1992a; 1992b).

Integrated weed management (IWM) is a holistic approach implementing one or more biological, physical, cultural or chemical control tactics (Harker and Donovan 2013). IWM aims to reduce weed adaptation and resistance to any single control tactic by using several possible tactics that take into account threshold populations, critical periods, and environmental outcomes. Utilizing IWM may lead to reduced environmental impacts of any given control method, decreased control costs by reducing pests to economically and ecologically insignificant levels, increased sustainability and reduced herbicide resistance (Harker and Donovon 2013). In a meta-analysis, Davis et al. (2018) found that combined treatments had better long-term outcomes for control of *C. arvense* than reliance on herbicide treatment alone. Mowing and tillage have been shown to affect *C. arvense* populations, but results have varied between significant reductions in population size to virtually no impact at all (Beck and Sebastian 2000; Bourdôt et al. 2011). A stem-mining weevil, *Hadroplontus litura*, and a bacterial plant pathogen, *Pseudomonas syringae* pv. *tagetis,* have been shown to have an additive effect in suppressing *C. arvense* treated with herbicide (Sciegienka 2011). *Puccinia suaveolens* has also previously been used in conjunction with other control methods. Mowing combined with *P. suaveolens* strongly reduced the proportion of fertile flower heads of *C. arvense* compared to infection alone (Kluth et al. 2003). Demers et al. (2006) found that systemically infected *P. suaveolens* shoots increased, while healthy shoots decreased when combined with mowing. In a greenhouse experiment with a crop sequence of winter wheat, spring pea and summer safflower, crop competition reduced *C. arvense* aboveground biomass but when inoculated with *P. suaveolens*, the effect was increased, by an approx. 10% (Chichinsky et al. 2023). Variable levels of control have also been observed across different environments, likely based on a combination of genetic and local conditions. A recent study by Bean et al (2024) saw stem densities declined at 77% of treated sites. The study also found that the pathogens effect was greater with increased inoculum, frequency and broadcast application (Bean et al. 2024). The potential of using *P. suaveolens* in an IWM approach for *C. arvense* is supported, but more research is needed to develop and refine best management practices.

Canada thistle is a problematic weed that is difficult to control, and current methods have varying degrees of success in both managed and natural ecosystems. As a biological control for *C. arvense*, *P. suaveolens* has significant potential, as it can self-perpetuate, spread to surrounding areas, and contribute to population suppression at large scales when applied alone (Bean et al. 2024). Our objectives were to determine the efficacy and compatibility of different control methods (mowing, tillage, herbicide, and *P. suaveolens*) when applied alone and in combination to suppress *C. arvense*. We highlight the benefits and limitations of using *P. suaveolens* in an IWM program, along with considerations for improved application efficacy.

Materials and Methods

Study Sites

Two experimental sites were established in 2020: one in the Tamarack Ranch State Wildlife Area of Colorado (CO, 40.8320°N, 102.80437°W) and the other in Park City, Utah (UT 40.674330°N, 111.491324°W). The CO site is within the High Plains Ecoregion, while the UT site is within the Wasatch and Uinta Range Ecoregion (Omernik and Griffith 2014). The CO site

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is a 12 by 600 m plot of land, characterized as a shortgrass prairie with seasonal water inundation. Historically, the site had been maintained as a food crop plot for wildlife. At the end of each growing season, glyphosate was applied, and the plot tilled for several years, resulting in the formation of a near monoculture of *C. arvense* (Levi Kokes, personal communication May 2020). The CO site typically has precipitation occurring throughout the year (Supplementary Table S:1). The UT site is a small preserve nestled within a suburban development. Historically, herbicides were applied, particularly for musk thistle (*Carduus nutans* L.), and goat/sheep grazing was occasionally employed at the UT site but had not occurred for many years; the area is largely left untouched (Sara Jo Dickens, personal communication, 2020). The UT site generally has most of the precipitation occurring in the winter months (S:1) (PRISM Climate Group 2023).

***Puccinia suaveolens* Inoculum**

Dried inoculum was prepared following Berner et al (2013) and Bean et al (2024). Briefly, *C. arvense* leaves bearing telia (small pustules on yellowing leaves), were collected in late summer from a site near Colorado Springs, CO. Leaves were harvested and stored in paper bags to allow foliage to dry at room temperature. Dried leaves were ground to a coarse powder in a kitchen blender and used as inoculum within the season or stored at -80 ◦C for future use. Samples of ground leaf preparations were viewed under a microscope to ensure most of the spores were two-celled teliospores, which are necessary for initiating systemic infection (French and Lightfield 1990; Berner et al 2013; Van Den Ende et al. 1987).

Experimental Design

In both UT and CO, an experiment site was established using a randomized complete block design, consisting of 10 treatment combinations applied across replicates. Treatments included an untreated control, *P. suaveolens* inoculation alone, tillage, tillage plus *P. suaveolens* inoculation, mowing, mowing plus *P. suaveolens* inoculation, herbicide (aminopyralid and chlorsulfuron tank mix), herbicide plus *P. suaveolens* inoculation, herbicide, mowing, and tillage (HMT), and HMT plus *P. suaveolens* inoculation (Table 1). Each treatment was applied once in 2020 and 2021 to field plots (CO: 2 by 6 m; UT: 2 by 5 m). Plots were spaced (CO:4m; UT:2m) apart to avoid edge effects with 8 replicates in CO and 4 in UT. Differences in experimental set up between sites were due to the size and accessibility of *C. arvense* populations.

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Herbicide and mowing treatments were applied in the fall during the first week of September. An herbicide tank mix was applied (aminopyralid 7 fl. oz/acre; chlorsulfuron at 1 fl. oz/acre) using a backpack sprayer calibrated in the field. Aminopyralid was chosen specifically as it is more effective at lower rates compared to other herbicides (e.g., picloram and clopyralid) and may also be used in areas where other chemicals are not appropriate or recommended (Enloe et al. 2017). In herbicide and mowing combination treatments, mowing was applied first to provide an opportunity for more even herbicide application and uptake given the physical damage to *C. arvense* (Carpinelli 2004). Fourteen days after initial treatments with mowing and herbicide, tillage (30 cm depth) and *P. suaveolens* inoculum (40 g CO, 33.3 g UT) were applied to select plots. When applying *P. suaveolens* inoculum, the entire plot was first sprayed with water using a backpack sprayer to create a mist on *C. arvense* leaves, then *P. suaveolens* was broadcast by hand no higher than 1m above the ground to avoid excessive dispersal by wind. The 14-day period allowed the herbicide to translocate through the roots and other tissues before tillage following recommended manufacturer (Corteva) guidelines. *Puccinia suaveolens* inoculum was applied last either alone or in combination (Table 1). The later timing for inoculum application in IWM treatments allowed for new growth and rosettes of *C. arvense* in response to mowing and possibly tillage, which may improve the chance for infection (Demers et al. 2006). Applications of *P. suaveolens* inoculum before mowing, tillage, or herbicide spray, would have been detrimental to germinating teliospores, which may have begun developing mycelia in the live tissue and subsequently been destroyed (Berner et al. 2013; Petersen 1974).

The initial monitoring of plots at both sites occurred in fall prior to first treatments. Monitoring occurred two weeks prior to the optimal timing for *P. suaveolens* teliospore inoculum application at each respective site. At both sites, a quadrat (m^2) was placed in the center of each plot and the number of thistle stems was counted and percent groundcover of *C. arvense*, grass, forbs, litter, and bare ground was estimated visually. Across the entire plot, a two-minute timed count of *C. arvense* stems systemically infected with *P. suaveolens* was also performed.

Statistical Analyses

All analyses were performed with R (R Core Team 2023), using packages tidyverse, ggplot2, glmmTMB, DHARMa, emmeans and car (Brooks et al. 2017; Fox and Weisberg 2019; Hartig 2022; Lenth 2024; Wickham et al. 2019). Data from the two sites were analyzed separately because of the imbalance in design due to the difference in site accessibility and size of *C. arvense* population. Stem count density change as a function of *P. suaveolens* inoculum application (Yes or No); management approach [Control, Herbicide (H), Mowing (M), Tillage (T), and HMT]; or year (Fall 2020, Fall 2021, and Fall 2022); and the interaction between combinations of these parameters were analyzed with a generalized linear model (GLM). Stem density was modeled (with negative binomial) also using GLM. Significance was tested using ANOVA type II Wald chi-square tests, followed by post-hoc pairwise Tukey test. Finally, ground cover data were analyzed using a GLM (with beta distribution) testing significance with ANOVA type II Wald chi-square tests, followed by post-hoc pairwise Tukey test. For all CO analyses, block 8 data was removed, as a Tukey's fence method test determined that the Herbicide and HMT plots were significant outliers. Block 8 lies in a section of the field that experiences significant seasonal water inundation and herbicide effects were likely diluted in their effects.

Results and Discussion

In this study, conducted in two regions of the western U.S., we evaluated the efficacy of *P. suaveolens* and its compatibility with other control methods for managing *C. arvense* by measuring stem density and vegetative cover. While stem density reflects the direct effects on the target weed, vegetative cover can represent biodiversity, forage availability, resiliency of the landscape, nutrient cycling, and may be used to predict production costs for livestock producers or fire risk. Consideration of both stem density and resulting vegetative cover will help land managers to make informed decisions about which treatments work in their IWM plan and how *P. suaveolens* can be incorporated.

Site Conditions and Stem Density

At the outset of the study, initial average stem density in CO was nearly four times that observed in UT (Figure 1). These differences may be attributed to prior management practices employed and variation in climate conditions experienced at each site (PRISM Climate Group 2023, Table S:1). The UT site has higher average annual precipitation compared to the CO site, although during the first year of treatments they were about equal (Table S:1). At the UT site, most precipitation occurs as winter snowfall, resulting in extended dry periods that can stress *C. arvense*, reducing root and stem growth, and subsequently, stem density and coverage (Tiley 2010). In contrast, Tamarack Ranch, CO, receives more evenly distributed precipitation

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throughout the year. At the UT site, temperature ranged from -11.1C during the coldest months to 29C in the hottest months of the experiment period (2020-2022). At the CO site, the temperature ranged from -13.5C during the coldest months to 33C (2020-2022) during the hottest months.

Herbicide and Herbicide+Mowing+Tillage (HMT)

Herbicide treatments, whether alone or in combination, were most effective in decreasing *C. arvense* stem density at both sites, (UT: $P<0.001$; CO: $P<0.001$). There was an immediate decline in stem density that continued even after the second application, with sparse regrowth observed (Figure 1). In CO, herbicide only treatments, stem density decreased 95% in year one and another 62% in year two. When *P. suaveolens* was applied along with herbicide, stem density declined 91% in year one and 100% the following year. The UT site experienced similar decreases in stem density caused by herbicide: year one 97%; and year two 100%. Herbicide plus *P. suaveolens* reduced stem density in year one by 98% and year two by 100% (Table 2).

In CO, the combined treatment (HMT) without *P. suaveolens* reduced stem density by 99% in year one and 85% in year two. When *P. suaveolens* was applied along with the HMT treatment, stem density decreased 85% in year one and 95% in year two (Table 2). At the UT site, stem density in the HMT plots without *P. suaveolens* decreased 69% in year one and another 95% in year two. When *P. suaveolens* was applied along with the HMT treatment, stem density decreased 84% in year one and 100% in year two (Table 2). Aminopyralid, which is selective against broadleaf weeds in rangelands and pastures, provided near 100% control of *C. arvense* in herbicide-treated plots with additive effects from *P. suaveolens* inoculum application. Limited thistle regeneration was observed, likely emanating from neighboring plants in buffer zones between plots, seeds, or remaining root fragments.

Puccinia suaveolens

Puccinia suaveolens was present at both sites in plots after treatments, however, there were only a few symptomatic stems. In CO, no symptomatic plants were found during the fall monitoring. In UT, the symptomatic shoots were found in the tillage plus *P. suaveolens* treatment, first appearing in year one (1 shoot) and also in year two (4 shoots). There was an overall lack of statistical significance (Table 3) of *P. suaveolens* impact at both sites, but a general declining trend in stem density indicating that the *P. suaveolens* had a slight suppressing effect on *C. arvense* (Figure 1, Table 2).

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In UT, *P. suaveolens* treatments alone appeared to slow the increase of stem density by 48% after two years when compared to the untreated control, which had a stem density increase of 98% (Table 2). In CO, *P. suaveolens* treatment, reduced stem density 22% more than the untreated control (Table 2). Bean et al. (2024), also observed *C. arvense* stem density decrease after *P. suaveolens* application at 77% of treated sites in Colorado over 3-8 years, stem density went from 87.9 ± 6.5 stems to 44.7 ± 4.2 stems on average. Sites with more frequent and higher quantities of *P. suaveolens* inoculum applied had a lower stem density over time. We suspect that *C. arvense* stem densities within *P. suaveolens* treated plots will continue to decrease with or without additional inoculations.

While stem decline was observed, the lack of symptomatic thistle stems could potentially be attributed to genotypic differences and associated resistance within *C. arvense*, or the compatibility of the host-pathogen interaction. Alternatively, the abiotic or biotic factors that induce production of systemically infected stems may not have been met, though vegetative mycelium within the root system could still be present. *Puccinia suaveolens* may continue to have an impact on *C. arvense* or additional inoculation treatments might be required. This could be the case at both sites, but particularly at the CO site, where stem density decline was more obvious in Fall 2022 after a second inoculation (Figure 1).

Mowing

There was no significance between mowing plots in CO, however a trend with mowing plus *P. suaveolens* showed a greater decrease in stem density (66%) compared to mowing alone (56%). In UT, mowing and mowing plus *P. suaveolens* resulted in a small decrease in stem density of 1% and 13% respectively with no significance.

The reduction in stem density between mowing with *P. suaveolens* inoculation and mowing alone was not statistically significant in UT or in CO. In CO, mowing with *P. suaveolens* inoculation initially had a smaller impact compared to mowing alone but still both decreased stem density (24% and 41%). However, in year two mowing with inoculations showed a greater decrease in stem density compared to mowing alone (55% and 26%) (Table 2, Figure 1). In UT, *C. arvense* stem density following mowing (averaged over *P. suaveolens*) was significantly lower compared to control (averaged over *P. suaveolens*), (UT: P=0.003, CO P=0.004). In CO mowing plus *P. suaveolens* was significantly lower than *P. suaveolens* alone (P=0.0178). In UT, mowing had significantly lower stem density (P=0.006) compared to tillage, with no significance in CO. Mowing has been used to enhance the occurrence of systematically infected stems (Bourdôt et al. 2011), and increases localized infection by spreading spores (Demers et al. 2006). Very few systemically infected stems were found during the 2-year study, which may have resulted in less additive effects from mowing with *P. suaveolens* compared to mowing alone. However, mowing should still be utilized with *P. suaveolens* in an IWM program, as the two treatments may be compatible and mutually beneficial based on reports of other studies.

Tillage

In CO, there was a significant difference (P<0.001, Table 3) between management practice and an interaction between management practice and season. Further analysis showed that tillage treatments had significantly greater decline in stem density compared to control in 2022 (P=0.009). There was no significant difference between tillage alone and tillage with *P. suaveolens* (UT: P>0.05, CO: P>0.05). The percent change in stem density is similar for both treatments: tillage in UT had an increase of 139% and in CO a decrease of 63%. In UT, tillage with *P. suaveolens* had a stem density increase of 142% and in CO a decrease of 70% (Table 2). In CO, tillage combined with *P. suaveolens* resulted in a slightly greater decrease in stem density in the first year (Figure 1) compared to tillage alone. The higher annual precipitation in the first year (Table S:1) may have contributed to *P. suaveolens* and tillage having a greater effect than in the second year. In our study, application of *P. suaveolens* inoculum did not cause a significant interaction with tillage but could be implemented in an IWM approach. Tillage has been used to manage *C. arvense* by reducing stem density through the depletion of root reserves and reduction in shoot biomass (Thomsen 2011; Weigel 2024). Proper timing of tillage can be crucial, as early tillage can allow *C. arvense* to recover and rebuild root reserves for overwintering (Donald 2000; Thomsen et al. 2015). Applying *P. suaveolens* inoculum two weeks after tillage may enhance pathogen invasion of the smaller root fragments, and increase systemic infection in the spring. (Alexopoulos et al. 1996; Berner et al. 2015a).

Groundcover

Cirsium arvense

The trends observed in *C. arvense* cover align closely with those in stem density. When cover measurements are divided by stem density, an estimate of the biomass of individual shoots can be made which may indicate the health of the population. Treatments with herbicide had the lowest amount of *C. arvense* cover, ranging from 0-25% in UT and 0-35% in CO. Of note, in

2022, plots treated with herbicide and *P. suaveolens* had zero *C. arvense* cover while plots treated with herbicide alone still had a low density of *C. arvense* stems. *Puccinia suaveolens* may have an additive effect in herbicide treated areas or help prevent regrowth, suggesting that these two treatments are compatible. Mowing also significantly reduced percent *C. arvense* cover compared to control, although no significant difference occurred between mowing alone and mowing with *P. suaveolens*. The greatest *C. arvense* cover occurred in the control, which was more easily seen in UT than in CO (Figure 2). Thistle cover was greater in tilled plots as a result of possible (not documented) fragmentation and spread of *C. arvense* roots creating many small populations (Donald 2000; Thomsen et al. 2015).

Vegetation

In UT, the herbicide treatment, which reduced broadleaf plants, including *C. arvense*, allowed more opportunity for grasses to grow (≤ 80% cover in year 2). Grass cover in UT was significantly higher in herbicide treatments compared to control and tillage (UT: $P<0.005$), with greater effects observed in the second year. In a three year study of management tactics for a non-native forb, a native grass cover increased as a result of herbicide treatments, however a steady and significant increase in non-native grass cover was also recorded (Skurski et al. 2013). Grasses were not identified to the species level and could include undesirable invasive species of concern (e.g., cheatgrass) for management of natural areas. In contrast, grass coverage in CO showed minimal change across treatments ($P>0.05$), though a significant interaction occurred between management and *P. suaveolens* ($P=0.003$). Further analysis showed that HMT and *P. suaveolens* treatments had significantly lower grass cover compared to control, *P. suaveolens*, or mowing plus *P. suaveolens* (Table 4; Figure 2). Tillage to 20cm distributes seeds throughout the entire tillage profile reducing accumulation of seeds near the surface, and therefore may have caused reduced germination and grass cover as seen in our results (Feledyn-Szewczyk et al. 2020). Differing climatic conditions between the two sites could also affect grass growth and contribute to the difference between treatments. Therefore, site characteristics need to be considered in conjunction with management strategy for potential revegetation or secondary invasion by non-native species.

Prior to initial treatments, forb cover was low (0-10%) and remained below 30% across most plots with no significant difference between treatments (UT: $P>0.05$, CO: $P>0.05$) (Table 4, Figure 2). Use of broadleaf herbicides against invasive forbs can be expected to also suppress

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both native and other exotic forbs within the treatment areas (Skurski et al. 2013). As expected, only treatments with broadleaf selective herbicides showed a slightly greater decline in forb cover. In other studies, short-term changes in native forb cover remained insignificant after herbicide application, except for reductions in flowering and seed set for at least 4 years post-treatment (Crone et al. 2009). There may be long-term implications in native forb recovery after herbicide is used to control non-native forbs like *C. arvense*.

Non-Vegetation

Bare ground significantly increased as a result of HMT treatments with and without *P. suaveolens* in UT ($P<0.001$). However, herbicide treatments with and without *P. suaveolens* did not result in a significant difference compared to other treatments ($P>0.05$). Combined treatments had more of an impact on bare ground cover in UT than herbicide alone perhaps due to significantly more grass cover in the herbicide alone treated plots in year two ($P<0.0001$). Combined control tactics have been shown in both cropping and non-cropping systems to have better long-term control of *C. arvense* than herbicide alone (Davis et al. 2018). There may have been an additional effect from changes in seedbank availability due to tillage (Feledyn-Szewczyk et al. 2020) or from mowing as mowing alone resulted in more bare ground compared to control ($P=0.03$). In CO, bare ground cover was significantly greater in both herbicide and HMT treated plots compared to all other treatments ($P<0.001$) (Figure 2). There was no significant difference found between bare ground cover in HMT and herbicide alone treated plots. Bare ground is an important aspect of land management since it may necessitate reseeding to prevent soil erosion and the creation of niches for other noxious weeds. Revegetation should be included to promote native and desirable plants (Molvar et al. 2024; Rodriguez et al. 2024; Weidlich et al. 2020).

At the UT site, herbicide treatments resulted in significantly more litter compared to tillage treatments (UT: $P=0.002$). In CO, HMT treatments had significantly lower litter compared to all treatments except for control (Table 4; Figure 2). Litter cover can help to retain soil moisture and increase nutrient cycling (Perera et al. 2024; Redman 1978)

Effective strategies for controlling *C. arvense* vary with site conditions, management goals, and operation budgets and the costs associated with the collection, processing, and distribution of *P. suaveolens* still needs to be evaluated. Chemical treatments are often the cheapest and most effective approach for reducing *C. arvense* populations but frequently increase areas of bare ground for invasion from other weeds, including re-invasion by *C. arvense*, as observed in our study. The use of herbicides is also often restricted in natural landscapes or on organically certified farms, leaving stakeholders in search of alternative and complementary control tactics. Applications of *P. suaveolens* inoculum in addition to other tactics such as mowing, show some promise for improved control of *C. arvense*. Continued monitoring will help determine if additional treatment applications are needed, especially where *P. suaveolens* effects are enhanced on *C. arvense*. Although *P. suaveolens* acts slowly to suppress *C. arvense*, one of the long-term benefits is that recovery of native plant communities is more likely. *Puccinia suaveolens* is unique among tactics for managing *C. arvense*, as there are no direct effects on non-target plants. Applying *P. suaveolens* alone or with other tactics may provide a safe and sustainable means to enhance management of *C. arvense* in some natural areas.

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Competing interests. The authors declare none.

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Table 1: Overview of weed management tactics employed for treatment of *Cirsium arvense* at experimental sites in Colorado and Utah.

Treatments	Equipment used	Application	Method	Optimal conditions and timing
Inoculation with P. suaveolens	Portable one-gallon spray tank	3.3g/m² inoculum	Wet all foliage with plain water, broadcast disperse inoculum, spray with water a second time.	Average daily temperature 65F Fall. Better with long dew periods such as evening. Added moisture layer. No wind
Herbicide (Chlorsulfuron and Aminopyralid tank mix)	Backpack 4-gallon manual piston-pump sprayer	Aminopyralid at 7 fl. oz/acre (0.11 acid equivalent), chlorsulfuron at 1 fl. oz/acre with non-ionic surfactant and marker dye tank mix	Broadcast spray plot directly after mowing if combined treatment.	Low wind, no rain, straight after mowing to help with translocation
Tillage	Honda 270cc Tiller Rear Tine 20"	Tilled to depth of 12 in.	Till 14 days post mowing or herbicide and	Best when with other methods. Fall best

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			directly preceding inoculations if combined.	timing. Earlier in the season requires more applications
Mowing	STIHL FS 91 16.5 in. Gas Trimmer	Mowed to height of 6 in.	Mow 2 weeks preceding inoculations.	Before seeding ideal

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Table 2: Annual average *Cirsium arvense* stem count change (%) and average stem count (m^2) with +/- standard error (SE) in Colorado and Utah from 2020-2022 following combined and individual treatments (MHT = Mowing+Herbicide+Tillage).

Treatment	Rust	Colorado						Utah					
		Stem count ± SE 2020	Stem count ± SE 2021	Stem count ± SE 2022	Fall 2020 2021 % change	Fall 2021 – 2022 % change	Fall 2020-2022 % change	Stem count ± SE 2020	Stem count ± SE 2021	Stem count ± SE 2022	Fall 2020-2021 % change	Fall 2021-2022 % change	Fall 2020-2022 % change
Control	No	83 ± 10	76 ± 11	65 ± 22	-9	-15	-22	22 ± 8	40 ± 5	42 ± 9	85	7	98
Control	Yes	97 ± 11	76 ± 15	54 ± 14	-21	-29	-44	26 ± 4	46 ± 4	38 ± 6	81	-19	46
Herbicide	No	82 ± 8	4 ± 1	1 ± 1	-95	-62	-98	29 ± 6	1 ± 1	0 ± 0	-97	-100	-100
Herbicide	Yes	92 ± 13	8 ± 4	0 ± 0	-91	-100	-100	22 ± 7	0 ± 0	0 ± 0	-98	-100	-100
Mowing	No	80 ± 15	47 ± 12	35 ± 11	-41	-26	-56	22 ± 5	26 ± 5	21 ± 5	21	-18	-1
Mowing	Yes	68 ± 15	52 ± 13	23 ± 10	-24	-55	-66	25 ± 8	30 ± 8	22 ± 4	18	-26	-13
MHT	No	72 ± 17	1 ± 1	0 ± 0	-99	-75	-100	25 ± 5	8 ± 3	1 ± 1	-69	-84	-95
MHT	Yes	91 ± 17	14 ± 6	1 ± 1	-85	-95	-99	27 ± 3	4 ± 3	0 ± 0	-84	-100	-100
Tillage	No	100 ± 12	69 ± 11	37 ± 15	-31	-47	-63	19 ± 3	43 ± 7	45 ± 5	128	5	139
Tillage	Yes	86 ± 15	40 ± 15	25 ± 9	-53	-36	-70	20 ± 3	43 ± 8	48 ± 9	118	11	142

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Table 3: Statistical results on the impact of the rust pathogen, management practice, and their combination across seasons on *Cirsium arvense* stem count in Colorado and Utah.

	Colorado			Utah		
	Chisq	Df	Pr(>Chisq	Chisq	Df	Pr(>Chisq
Rust	0.0378	1	0.8459	0.0153	1	0.9017
Management	96.4278	4	<0.0001	60.6082	4	<0.0001
Season	218.6041	2	<0.0001	0.7936	2	0.6725
Management x Rust	12.2471	4	0.0156	3.1770	4	0.5286
Rust x Season	1.9095	2	0.3849	1.1180	2	0.5718
Management x Season	196.4279	8	<0.0001	170.0956	8	<0.0001
Management x Rust x Season	20.3150	8	0.0092	2.2153	8	0.9737

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Table 4: ANOVA table of the five ground cover types measured in UT and CO sites as a function of rust inoculum application, management strategy, season and the combined effects of these three parameters.

		Colorado										Utah									
		Cirsium arvense		Grass		Forbs		Litter		Bare Ground		Canada Thistle		Grass		Forbs		Litter		Bare Ground	
	Df	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq	Chi sq	Pr(>Chi sq
Rust	1	0.099	0.75	0.03	0.87	0.002	0.96	0.15	0.70	0.02	0.90	1.25	0.26	0.49	0.49	0.97	0.33	0.002	0.96	0.08	0.78
Management	4	31.31	<0.0001	6.09	0.19	5.44	0.25	23.62	<0.0001	55.90	<0.0001	62.73	<0.0001	21.33	0.0003	2.80	0.59	14.53	0.006	33.02	<0.0001
Season	2	222.99	<0.0001	76.02	<0.0001	3.09	0.21	147.71	<0.0001	26.61	<0.0001	21.07	<0.0001	95.70	<0.0001	0.28	0.87	60.23	<0.0001	1.06	0.59
Management x Rust	4	11.16	0.025	16.09	0.003	3.86	0.43	5.78	0.22	3.66	0.45	1.28	0.86	1.27	0.87	0.20	1.00	1.81	0.77	6.90	0.14
Rust x Season	2	2.22	0.33	0.11	0.95	0.50	0.78	0.51	0.78	0.01	0.995	0.05	0.9773	5.09	0.08	0.18	0.92	1.66	0.44	0.49	0.78
Management x	8	42.90	<0.0001	9.36	0.31	31.02	<0.0001	47.83	<0.0001	116.33	<0.0001	222.42	<0.0001	77.90	<0.0001	9.20	0.33	13.39	0.10	53.18	<0.0001

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Season																					
Management x Rust x Season	8	2.29	0.97	4.41	0.82	11.58	0.17	10.84	0.21	4.62	0.80	8.41	0.39	26.93	0.0007	6.03	0.64	8.59	0.38	8.61	0.38

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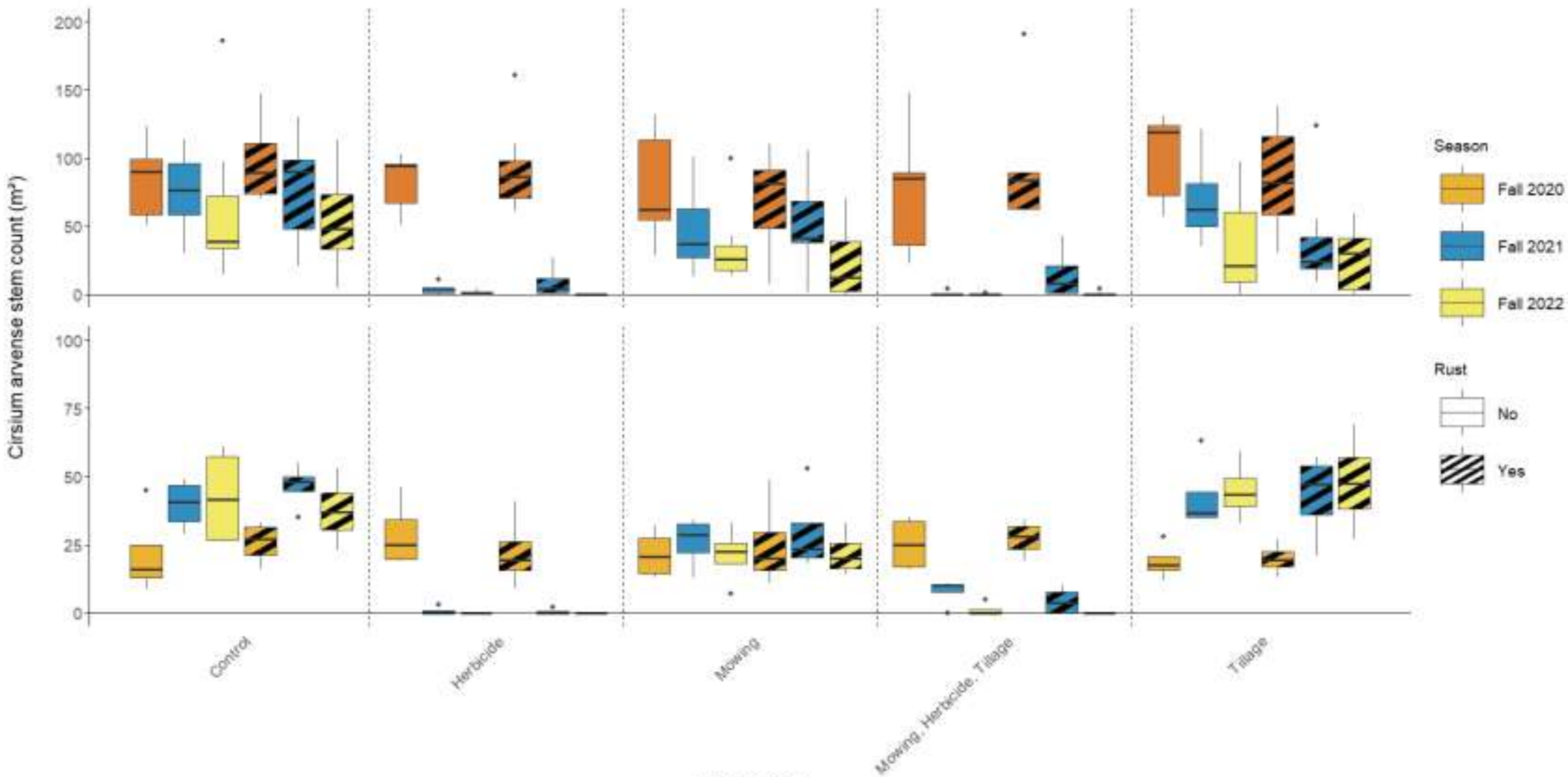

Figure 1: *Cirsium arvense* stem count (m^2) in Fall 2020-2022 in A) Colorado and B) Utah following treatment with individual and combined weed management approaches.

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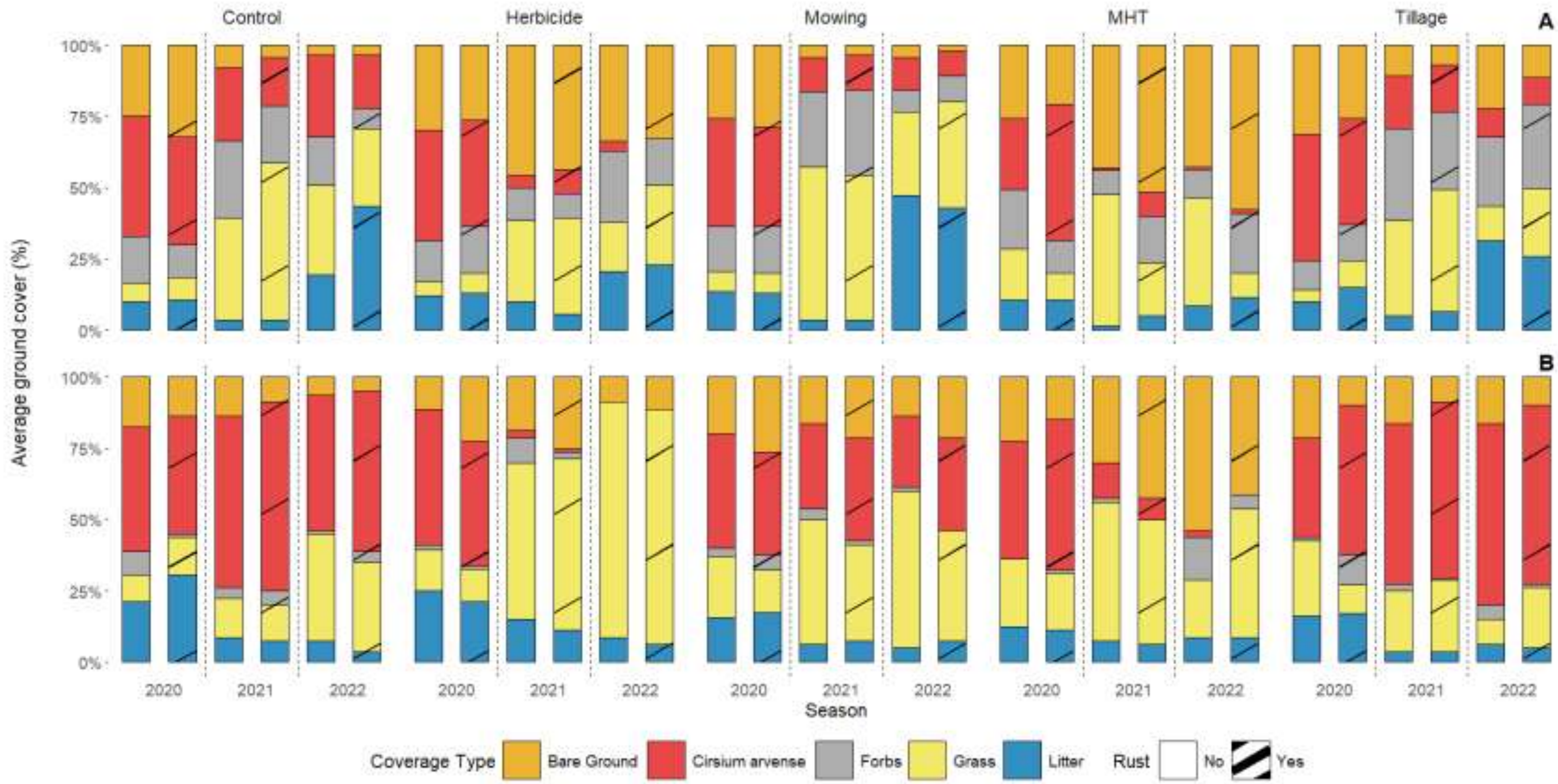

Figure 2: Average percent of the 5 measured ground cover types. A) Colorado and B) Utah experimental site, 2020-2022 following treatment with individual and combined weed management approaches.

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