

Integrating *Puccinia punctiformis*, a biological control agent, into *Cirsium arvense* management in semi-arid organic agriculture

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Integrating *Puccinia punctiformis*, a biological control agent, into *Cirsium arvense* management in semi-arid organic agriculture

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HIGHLIGHTS

- *P. punctiformis* aids management of *C. arvense* in organic agroecosystems.
- Reduced tillage increased *P. punctiformis* occurrence and symptomatic stem density.
- Reduced tillage – *P. punctiformis* combination reduced *C. arvense* stem density.
- Reduced tillage and *P. punctiformis* decreased *C. arvense* relative growth rate.
- Crop response was positive to *P. punctiformis* presence under reduced tillage.

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ABSTRACT

Puccinia punctiformis is an obligate fungal pathogen of *Cirsium arvense*, a perennial rhizomatous weed common in temperate ecosystems. *Cirsium arvense* is difficult to manage in organic agriculture and integrated management is necessary to reduce its abundance and impact on crops. *Puccinia punctiformis* limits *C. arvense* growth but its effect has not been quantified in cropland field studies. In a four-year organic annual crop sequence at a semi-arid site in Montana, USA, we evaluated (1) *P. punctiformis* (occurrence, symptomatic stem density) and *C. arvense* (stem density, relative growth rate [RGR]) in response to tillage (standard vs. reduced), and the relationship between these two variables, and (2) final year crop stem density in response to tillage and *P. punctiformis* patch infection. Reduced tillage increased *P. punctiformis* occurrence and symptomatic stem density through time. *Cirsium arvense* stem density decreased through time in reduced tillage patches that displayed symptomatic infection. *Cirsium arvense* RGR decreased with increasing years that a patch had *P. punctiformis* symptomatic infection, and in the reduced tillage treatment regardless of symptoms. Final year crop stem density was higher under standard tillage but increased under reduced tillage with increasing years that a patch had *P. punctiformis* symptomatic infection. Reduced tillage practices complemented the impact of existing *P. punctiformis* by increasing its spread and symptomatic infection, two challenges previously identified with using *P. punctiformis* as a biocontrol agent. When coupled with complementary tactics, producers can integrate *P. punctiformis* into semi-arid organic agriculture weed management, potentially helping reduce *C. arvense*'s impact on crops.

1. Introduction

Integrated weed management combines mechanical, cultural, biological, and chemical control measures to manage weeds (Esposito et al., 2023; Westerman et al., 2005). Using an ecological approach that focuses management at different life-cycle stages, integrated weed management aims to shift weed communities that are dominated by a few highly competitive species towards more diverse communities that have less impact on crops (Liebman et al., 1997). To be successful, integrated

weed management must be tailored to the biological and ecological characteristics of the crop-weed complex, and consider the interactions among different management practices (Begg et al., 2017; Harker & O'Donovan, 2013).

Conservation biological control focuses on promoting populations of natural enemies (e.g., pathogens, predators, parasitoids) already present in the system (Begg et al., 2017; Pell et al., 2010), with potential to be used in agricultural settings (Landis et al., 2000). When a natural enemy is present but ecologically extinct in a system, conservation biological

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control manipulates the environment to enhance its survivorship and impact (Eilenberg et al., 2001; Stenberg et al., 2021). In agroecosystems, conservation biological control has largely focused on providing resources for beneficial invertebrates (e.g., ‘beetle banks’; Barbosa, 1998; Gontijo, 2019), and biological control using fungal pathogens has largely been inundative (i.e., mycoherbicides). Non-inundative (i.e., classical, conservation) pathogenic biological control of weeds in agroecosystems is difficult because management practices can adversely affect the biocontrol agent (Begg et al., 2017; Charudattan & Dinooor, 2000). For example, the type and timing of management (e.g., cultivation, tillage, mowing, chemical) can limit spore distribution and pathogen transmission (Termorshuizen, 2017), neutralize the pathogen (e.g., fungicides; Letourneau, 1998; Orr and Fox 2012), and decrease the habitat suitability for the persistence of the pathogen (Newman et al., 1998).

Cirsium arvense (L.) Scop. (Canada, Californian, or creeping thistle) is a perennial weed that spreads through extensive underground rhizomatous lateral roots with buds that can form new clonal plants and wind dispersed seeds (Tiley, 2010), and is one of the most problematic pests in semi-arid organic cropping systems (Orloff et al., 2018; Tautges et al., 2016). In these systems, mechanical management, specifically cultivation or tillage, is the most commonly used practice, and when done at high frequencies can be effective for its management (Mohler, 2001). However, tillage decreases soil stability and structure (Hettinger et al., 2023), increasing wind and water erosion, and soil moisture loss through evaporation (Lehnhoff et al., 2017; Triplett Jr & Dick, 2008). These ecological impacts make finding effective no-till or reduced-till weed management options for semi-arid organic systems particularly important (Lehnhoff et al., 2017). Cultural methods (i.e., perennial forage crops; Gramig et al., 2024; Larson et al., 2024) have been effective, however integrated management has been proposed as the best approach (Davis et al., 2018; Orloff et al., 2018). Using fungal pathogens in combination with herbicides has increased *C. arvense* control (Bailey et al., 2000), and there is potential for integrating fungal biocontrol agents into organic management plans (Burns et al., 2013; Cripps et al., 2011; Davis et al., 2018; Sciegienka et al., 2011).

Puccinia punctiformis (F. Strauss) Rohl. is an obligate heterotrophic fungal pathogen of *C. arvense* that acts as a long-term systemic parasite. It is believed that *P. punctiformis* was introduced to North America with *C. arvense*, and it is now naturalized worldwide (Bradshaw et al., 2023). The pathogen primarily exists as a root parasite, but can impact above ground growth, lowering fitness and causing death (Berner et al., 2013; Watson & Keogh, 1981). The fungus matures and produces transmissible spores (‘teliospores’) on above ground *C. arvense* tissue during summer months (Berner et al., 2013). In the fall, the infected tissue senesces, abscises/disperses, and can contact and infect healthy *C. arvense* rosettes (Berner et al., 2013; French & Lightfield, 1990). In rangeland and non-cropping systems, the impact of *P. punctiformis* on *C. arvense* abundance is well documented (Bean et al., 2024; Berner et al., 2015; Watson & Keogh, 1981). Likewise, in controlled experiments, *P. punctiformis* has worked additively with competing species to reduce *C. arvense* above-ground and belowground biomass (Chichinsky et al., 2023), and with simulated mowing to reduce seed production (Kluth et al., 2003).

Enhancing *P. punctiformis*’ effectiveness as part of a conservation biological control program to manage *C. arvense* in agricultural systems has not been assessed in field studies. To address this knowledge gap, *P. punctiformis* symptomatic infection and *C. arvense* were evaluated across a four-year organic annual crop sequence in response to different tillage regimes (standard vs. reduced tillage). Asymptomatic *P. punctiformis* infection does occur, however, previous work has demonstrated that *P. punctiformis* symptomatic/systemic infection is a good indicator of overall patch infection (Berner et al., 2015). Our research questions were: 1) Can different tillage regimes (reduced vs. standard) be used as conservation biological control strategies to increase the occurrence of *C. arvense* stems displaying symptoms of *P. punctiformis* infection through time? 2) In those patches where

symptomatic infection is observed, does tillage affect the density of *C. arvense* stems that have symptomatic infection through time? 3) What is the *C. Arvense* stem density response to the combined effect of tillage and the presence of symptoms within a patch (‘symptomatic’ vs. ‘asymptomatic’) through time? 4) Is there a relationship between the observed number of years that a patch demonstrated symptomatic infection during the study and *C. arvense* relative growth rate, and does this relationship differ with tillage? 5) How does the final year crop response (stem density) differ as a function of tillage, and the observed number of years that a patch had symptomatic infection during the study?

2. Materials and methods

2.1. Study area and experimental design

The study took place across four years (2020 – 2023) at the Montana State University Fort Ellis Research Farm (45.667137° N, –110.977948° W). Fort Ellis is a non-irrigated farm approximately 6 km east of Bozeman, Montana. The site has a 1.5 m deep Blackmore silt loam soil profile (Soil Survey Staff (NRCS), 2023), the mean annual precipitation during the study period was 530 mm, and the mean annual temperature was 6 °C (PRISM Climate Group, 2023). The study area was USDA organically certified in 2015 and hosted reduced-till cropping studies until 2017, details can be found in Larson et al. (2021). It remained fallow in 2018, followed by a year of forage barley in 2019.

The study used a split-plot design, with plots replicated four times, and each separated into two split-plots (~ 45 m x 13 m) randomly assigned to tillage treatment (standard or reduced). The four standard tillage split-plots had three preexisting *C. arvense* patches (n = 12), while two of the reduced tillage split-plots had three preexisting patches and two had four patches (n = 14; Supplementary Fig. 1). Half of the patches in both tillage treatments had *C. arvense* stems with preexisting signs/symptoms of *P. punctiformis* infection. Patches were considered discrete when there was at least two meters of distance between patch boundaries, which is similar to other studies (2.5 m, Weigel et al., 2023) but less than others (10 m, Eber & Brandl, 2003). In June 2020, initial *C. arvense* patch boundaries were mapped as polygons with an Emlid Reach RS2 + GNSS receiver. Average patch size did not differ between standard and reduced tillage split-plots ($F_{(1,20.97)} = 1.77$, $P = 0.197$). GPS polygon data from 2020 were used to relocate patches throughout the study.

2.2. Management

All plots were uniformly cultivated with a chisel plow in the fall of 2019 and were drill-seeded into the first year of a four-year cover crop sequence in spring 2020. The studied crops are commonly grown in the region by organic farmers: (2020) 90 kgs/hectare of forage barley (*Hordeum vulgare* L.); (2021) 91 kgs/hectare of winter pea (*Pisum sativum* L.) intercropped with 84 kgs/hectare winter triticale (*X tritosecale*); (2022) 90 kgs/hectare of spring wheat (*Triticum aestivum* L.); (2023) 90 kgs/hectare of spring wheat.

The standard tillage treatment used a tandem disc harrow (15-cm depth) and a chisel plow (15-cm depth) for weed management, crop termination, and seedbed preparation. The reduced tillage treatment used a flail mower (183-cm width) set to leave 10-cm stubble for crop termination, weed management, and seedbed preparation. After mowing, seedbed preparation in the reduced tillage treatment used light surface harrowing (1.5-cm depth). For years one and two, tillage treatments occurred in the fall, while in years three and four, they were implemented in the spring immediately prior to planting spring annual crops.

2.3. Sampling methods

In each *C. arvense* patch, data collection used randomly placed 1-m² quadrats and was timed with the development of *P. punctiformis* teliospores on infected stems (July). The number of quadrats per patch was scaled based on patch size, with sample sizes ranging from three quadrats in small patches to nine quadrats in large patches. Within each quadrat, the densities of asymptomatic and symptomatic *C. arvense* and the crop (stem count · m⁻²) were assessed. *Cirsium arvense* stems were considered symptomatic when they had visible signs of *P. punctiformis* infection and displayed spores on aboveground structures. In addition, the total density of symptomatic stems in each patch (stems · patch⁻¹) was assessed yearly by walking the entire patch and recording/counting all stems that displayed visible signs of spore formation (most likely teliospores but all stages of spore development were considered symptomatic).

2.4. Statistical analysis

The probability of *Puccinia punctiformis* infection was estimated using a logistic regression generalized linear mixed-effects model with a binomial distribution ('glmer', R-package 'lme4'; Bates et al., 2015), with a response of presence/absence of stems with symptomatic infection, fixed effects of tillage treatment, year (factor), the interaction between the variables, the covariate of 'initial symptoms,' and a random effect of unique patch identity. A patch was considered to have 'initial symptoms,' when it had visible signs of spores and symptomatic infection in the first year of the study (2020).

Using only data from those patches with symptomatic infection, total symptomatic stem density data were analyzed using a generalized linear mixed-effects model with a negative binomial distribution ('glmer.nb', R-package 'lme4'; Bates et al., 2015) with fixed effects of tillage treatment, year (factor), the interaction between the variables, the covariate of initial symptoms, and the random effect of patch identity. A negative binomial model was used to alleviate statistical issues with overdispersion.

Cirsium arvense stem density (hereafter 'stem density') data were analyzed using a generalized linear mixed-effects model with a negative binomial distribution (Bates et al., 2015), with fixed effects of a flattened term that combined tillage treatment and presence of symptoms in the patch ('symptomatic' or 'asymptomatic' with *P. punctiformis* infection), year (factor), the interaction between the two variables, the covariate of initial symptoms, and a random effect of patch identity. We used the flattened term because we were interested in the combined effect of the tillage treatments and symptomatic infection on the response through time.

Patch relative growth rate (RGR) was derived using the average stem density for each patch in years one and four using the following equation:

$$RGR = \frac{\ln(\text{yr 4 stem density}) - \ln(\text{yr 1 stem density})}{(4 - 1)}$$

RGR was analyzed using a linear model with explanatory variables of the number of years a patch was recorded with symptomatic stems (numeric), tillage treatment, the interaction between the two variables, and the covariates of initial symptoms and plot. An extra-sum-of-squares F-test stepwise backward model selection process was used for model refinement, and the covariate of initial symptoms was removed from the final model. A type-III F-test was performed to determine if explanatory variables accounted for differences in the RGR response. Averaged across the covariate of plot, relative growth rate estimated marginal means ("emmeans", R-package "emmeans"; Lenth 2023) were derived from the model and compared between tillage treatments. To determine if the RGR of the tillage treatments differed from zero, estimated marginal means were tested against the default of zero using a reference grid and the "test" function of the R-package "emmeans" (Lenth, 2023). The

absence of highly influential observations was ensured using cook's distance (>1), and the assumptions of linearity, homoscedasticity, and normality were visually assessed, and a quadratic term of 'number of years symptomatic' was used to reduce the evidence against the assumption of linearity.

Final year crop density data, averaged at the patch level, were analyzed using a linear mixed-effects model with fixed effects of the number of years a patch was recorded with symptomatic stems (numeric), tillage treatment, the interaction between the two variables, the covariates initial symptoms and *C. arvense* stem density, and plot as a random effect. An extra-sum-of-squares F-test stepwise backward model selection process was used for model refinement, and the covariates of initial symptoms and *C. arvense* stem density were removed from the final model. To determine if the crop density response to the number of years a patch was recorded with symptomatic stems differed from zero (no change), the estimated slopes of the tillage treatments, obtained using the 'emtrends' function of the R-package "emmeans" (Lenth, 2023), were compared against the default of zero using the 'test' function from the "emmeans" R-package.

For all temporal models, estimated marginal means were calculated from their respective models and were compared using Tukey post-hoc tests ('emmeans', R-package 'emmeans'; Lenth 2023). For all generalized linear mixed-effects models, type-III Wald chi-square tests were performed to determine if explanatory variables accounted for differences in the responses ('Anova' in R package 'car'; Fox & Weisberg 2019). All analyses were conducted using the statistical software 'R' (R Core Team, 2024).

3. Results

3.1. *Puccinia punctiformis* infection probability of occurrence

Tillage and year interacted on the probability of occurrence of *C. arvense* with visible symptoms of *P. punctiformis* infection ($\chi^2 = 23.13$, $P < 0.001$). In the first year of the study (2020), the mean ($\pm$ SE) probability of occurrence of symptomatic *P. punctiformis* infection did not differ between the standard (0.22 ± 0.07) and reduced tillage (0.14 ± 0.05 ; $P = 0.35$; Fig. 1) treatments. Over the course of the study, the mean ($\pm$ SE) probability of symptomatic *P. punctiformis* infection did not differ in the standard tillage treatment, however it increased in the reduced tillage treatment, with a higher probability of occurrence in 2022 (0.58 ± 0.10) and 2023 (0.38 ± 0.09) compared with 2020 ($P <$

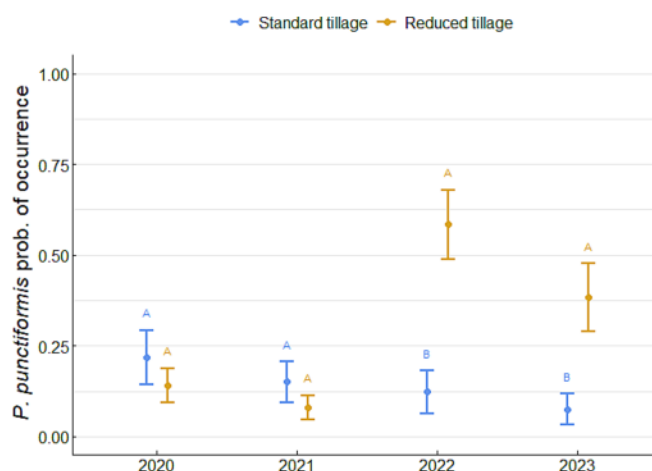


Fig. 1. Probability of occurrence of *Cirsium arvense* stems with visible symptoms with *Puccinia punctiformis* infection in the standard (blue) and reduced tillage (yellow) across the four years of the study (2020 – 2023). Means ($\pm$ SE) are back-transformed estimated marginal means derived from the generalized linear mixed-effects model. Letters above error bars indicate differences between tillage treatments within each year ($P < 0.05$).

0.001, $P < 0.029$, respectively) and 2021 (0.08 ± 0.03 ; $P < 0.001$, $P = 0.001$, respectively; Fig. 1). The different temporal trends resulted in a higher probability of symptomatic infection in the reduced compared with standard tillage treatment in both 2022 and 2023 ($P < 0.001$, $P = 0.005$, respectively; Fig. 1). Averaged over year and tillage, patches that had initial symptoms had a higher probability of symptomatic infection (0.32 ± 0.06) compared with initially asymptomatic patches (0.10 ± 0.03 ; $P < 0.001$).

3.2. *Puccinia punctiformis* symptomatic stem density

There was an interaction of tillage treatment and year on total patch symptomatic density (stems·patch⁻¹; $\chi^2 = 14.64$, $P = 0.002$). In 2020, mean ($\pm$ SE) *C. arvense* stem density with visible symptoms of infection was similar between the standard (5.78 ± 2.09) and reduced tillage (4.96 ± 1.67 ; $P = 0.751$; Fig. 2) treatments. Over the course of the study, symptomatic density did not differ among years under standard tillage, however it increased through time under reduced tillage. Reduced tillage symptomatic density was higher in 2022 (19.67 ± 5.03) compared with 2020 ($P < 0.001$), and trending towards a difference with 2021 (9.45 ± 2.8 ; $P = 0.085$), and was higher in 2023 (25.87 ± 6.91) compared with 2020 and 2021 ($P < 0.001$, $P = 0.014$, respectively). The different temporal trends of the tillage treatments resulted in higher symptomatic density in reduced compared with standard tillage by year 2023 ($P < 0.001$; Fig. 2). There was also higher symptomatic density in those patches that had initial symptoms (14.59 ± 2.81), compared with those patches that did not have initial symptoms (6.04 ± 1.48 ; $\chi^2 = 8.37$, $P = 0.004$).

3.3. *Cirsium arvense* stem density

Across the four years of the study, *C. arvense* stem density (stems·m⁻²) differed among the combinations of tillage treatment and *P. punctiformis* symptoms ('asymptomatic' vs. 'symptomatic'; $\chi^2 = 21.96$, $P = 0.009$). In 2020, there was a trend that mean ($\pm$ SE) *C. arvense* stem density was higher in symptomatic standard tillage patches (76.0 ± 7.94) compared with asymptomatic standard tillage patches (53.1 ± 6.55 , $P = 0.062$). There were no other differences among the combinations, except in 2023. In 2023, mean stem density of the symptomatic reduced tillage patches (46.8 ± 4.23) was lower than that of the symptomatic standard tillage patches (68.3 ± 6.91 , $P = 0.027$), and

there was a trend that it was lower than the asymptomatic standard tillage (72.7 ± 11.68 ; $P = 0.079$). Also in 2023, there were trends that the asymptomatic reduced tillage (40.2 ± 7.84) stem density was lower than the symptomatic standard tillage ($P = 0.074$) and the asymptomatic standard tillage ($P = 0.088$).

Temporal analysis of *C. arvense* stem density in each of the tillage-symptom levels demonstrated that the symptomatic reduced tillage patches had higher densities in 2020 (66.0 ± 6.26) and 2022 (60.6 ± 5.49) compared to 2021 (41.8 ± 3.66 ; $P < 0.001$, $P < 0.001$, respectively) and 2023 (46.8 ± 4.23 ; $P = 0.004$, $P = 0.041$, respectively; Fig. 3). There were no other notable differences through time, except in 2021, when low growing season precipitation resulted in low stem densities in all patches regardless of symptoms and tillage (Fig. 3).

3.4. *Cirsium arvense* relative growth rate

Cirsium arvense patch relative growth rate (RGR) declined the longer it remained symptomatic ($F_{2, 19} = 5.41$, $P = 0.014$; Fig. 4A). Mean ($\pm$ SE) patch RGR was higher under standard (-0.007 ± 0.056) compared with reduced tillage (-0.13 ± 0.05 ; $F_{1, 19} = 4.82$, $P = 0.041$; Fig. 4B). Standard tillage RGR did not differ from a neutral growth rate ($P = 0.899$), while RGR was negative under reduced tillage ($P = 0.018$).

3.5. Crop stem density

The interaction between the number years a patch was symptomatic and tillage treatment affected final year (2023) crop density (stems·m⁻²; $F_{1, 18.09} = 7.45$, $P = 0.014$; Fig. 5). Under standard tillage, there was no change in mean ($\pm$ SE) crop density for every year a patch was recorded with symptoms ($P = 0.814$). In the reduced tillage treatment, mean ($\pm$ SE) crop density increased for every year a patch was recorded with symptoms ($P = 0.005$).

4. Discussion

This study demonstrated that integrated weed management in organic agroecosystems requires complementary practices. When we integrated mechanical and biological practices for *C. arvense* management, we found that the mechanical component (standard vs. reduced tillage) differentially impacted the biological (*P. punctiformis*) component, which shaped their combined effect. Over the course of the four-year study, *P. punctiformis* symptomatic infection occurrence and density increased under reduced tillage and worked additively with the mechanical component to reduce *C. arvense* populations. Whereas the standard tillage treatment, characterized by more soil and root disruptive practices, did not complement *P. punctiformis*, and the overall effectiveness of the combined practices suffered as a result.

A major challenge to implementing conservation biological control in agroecosystems is integrating practices that promote target host susceptibility and alter the environment to increase biocontrol agent survival and enhance its impact (Charudattan & Dinooor, 2000; Kennedy & Kremer, 1996; Morin, 2020). These concerns directly apply to *P. punctiformis*, as facilitating widespread systemic infection is a limitation to its use and effectiveness as a biocontrol agent (Müller & Nentwig, 2011). Compared with standard tillage, we found that reduced tillage facilitated greater spread of *P. punctiformis* infection. It is likely that the reduced tillage treatment better fulfilled criteria necessary for effective biocontrol by promoting pathogen survival through increasing opportunity for infection and providing more favorable conditions for infection.

The over-winter survival of pathogens is one of the most significant challenges to their use as biocontrol agents for perennial weeds (Morin, 2020). The reduced tillage treatment may have increased *P. punctiformis* survival by enhancing the opportunity for infection by spreading spores more evenly across the field. In a non-cropland setting, Demers et al. (2006) observed an increase in *P. punctiformis* symptomatic infection of

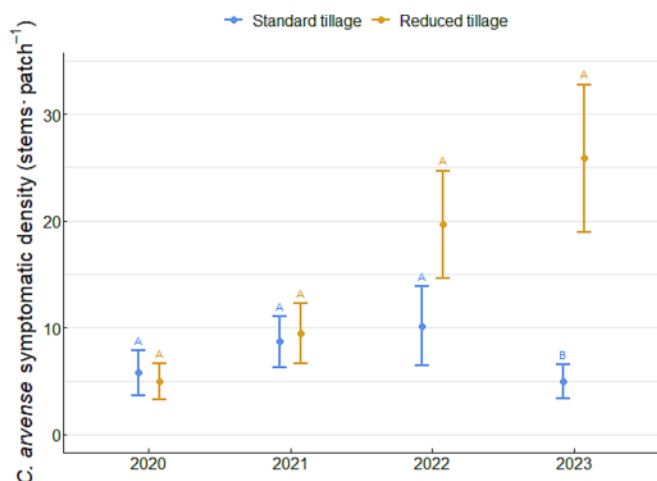


Fig. 2. Density of *Cirsium arvense* stems with visible symptoms of *Puccinia punctiformis* infection in the standard (blue) and reduced-tillage (yellow) treatments across the four years of the study (2020–2023). Means ($\pm$ SE) are back-transformed estimated marginal means derived from the negative binomial mixed-effects model. Letters above error bars indicate differences between tillage treatments within each year ($P < 0.05$).

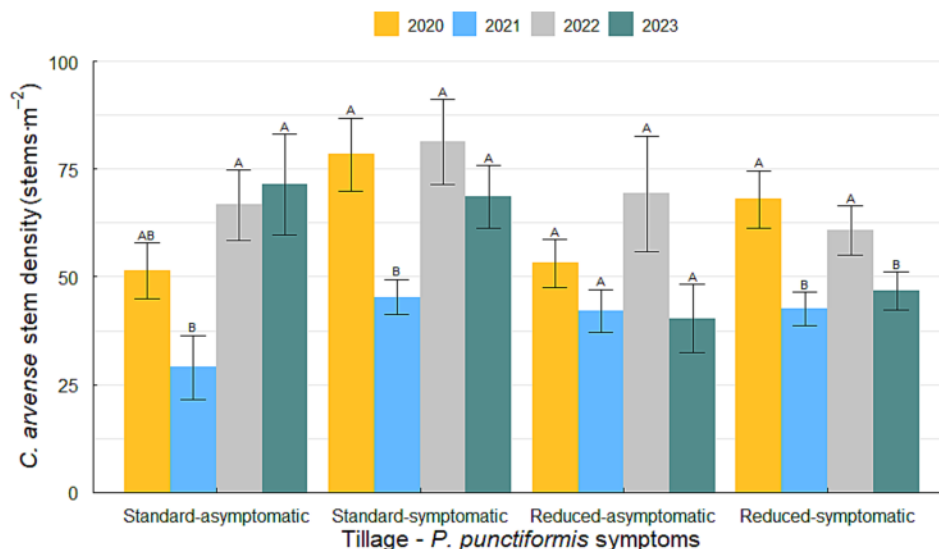


Fig. 3. *Cirsium arvense* stem density in response to the combination of tillage treatment (standard vs. reduced) and *Puccinia punctiformis* symptoms ('asymptomatic' vs. 'symptomatic') across the four years of the study (2020 – 2023). Means ($\pm$ SE) are back-transformed estimated marginal means derived from the negative binomial mixed-effects model. Letters above error bars indicate the differences among years for each combination of tillage treatment and patch symptoms ($P < 0.05$).

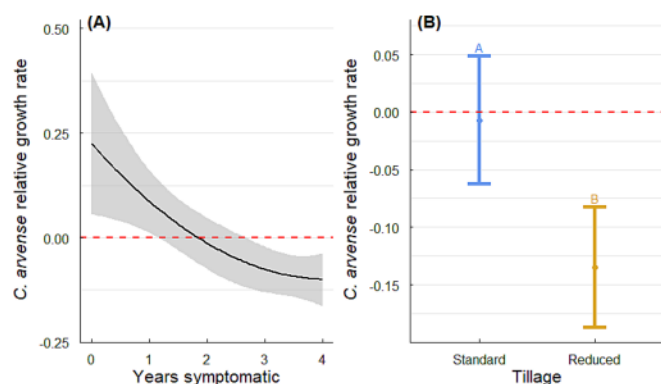


Fig. 4. *Cirsium arvense* relative growth rate (RGR) in response to (A) the number of years a patch displayed symptoms of *Puccinia punctiformis* infection, and (B) standard (blue) and reduced tillage (yellow) treatments. The red line denotes an RGR of 0. Letters above error bars indicate the difference between tillage treatments ($P < 0.05$).

C. arvense following mowing combined with the presence of symptomatic infection and concluded that mowing assists pathogen movement by uniformly distributing teliospores. This contrasts with standard tillage, which inverts soil A-horizons, potentially displacing and burying transmissible spores, thereby lowering pathogenic infection rates (Bockus & Shroyer, 1998; Rawat et al., 2021; Termorshuizen, 2017).

Berner et al. (2013) identified the optimal environmental conditions (i.e., humidity – temperature [dew point]) for *P. punctiformis* inoculation/infection to occur on *C. arvense* rosettes. Compared with standard tillage, reduced tillage leaves more plant residue on the soil surface, creating microclimate conditions favoring the persistence and germination of fungal pathogens (Bockus & Shroyer, 1998; Rawat et al., 2021; Wang et al., 2020). Furthermore, mowing *C. arvense* aboveground structures stimulates the production of stress and defense hormones, which has been linked with higher incidence of *P. punctiformis* symptoms (Clark et al., 2020). The combination of favorable microclimates and hormone production likely created conditions favorable for increased *P. punctiformis* infection. In contrast, standard tillage incorporates crop residue into the soil, results in drier soil surface conditions compared with reduced tillage (Rajanna et al., 2022), and damages *C. arvense*

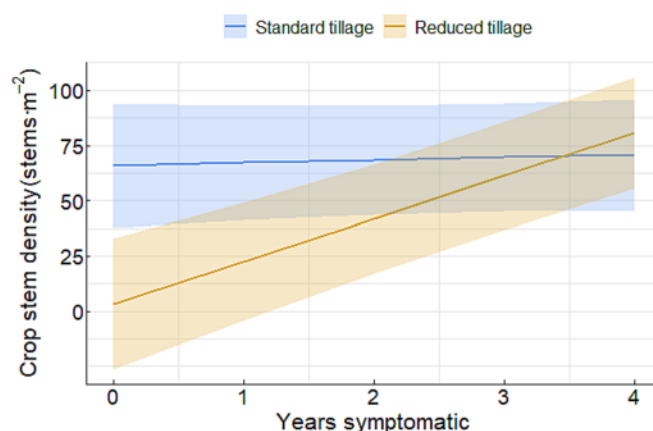


Fig. 5. Final year crop density (stems · m⁻²) response to the number of years a patch was recorded to have symptoms of *Puccinia punctiformis* infection under standard (blue) and reduced (yellow) tillage. Estimated slopes ($\pm$ SE) were derived from the crop density linear mixed-effects model.

roots. Consequently, standard tillage provides fewer microrefugia for pathogens (Bockus & Shroyer, 1998; Rawat et al., 2021), and may limit *P. punctiformis* transmission by decreasing its access to resources in damaged *C. arvense* rhizomes (Berner et al., 2015).

The observed relationship between *P. punctiformis* symptomatic infection and a decreasing *C. arvense* patch RGR and, when combined with reduced tillage, declining asymptomatic stem density are consistent with *P. punctiformis*' impact on *C. arvense* in non-cropland research (Bean et al., 2024; Berner et al., 2015; Chichinsky et al., 2023; Kluth et al., 2003; Watson & Keogh, 1981). Individually, repeated mowing can suppress *C. arvense* (Bickler & Masiunas, 2009; Bourdôt et al., 2016; Lukashyk et al., 2008) by depleting stored root carbohydrates that are then used for shoot regrowth (Graglia et al., 2006). As *P. punctiformis* primarily exists as a root pathogen (Berner et al., 2015; Kentjens et al., 2024), and can impact root biomass (Chichinsky et al., 2023; Clark et al., 2020), it is not surprising that the presence of symptomatic infection worked additively with the reduced tillage treatment to impact *C. arvense*.

The goal of biological control in agroecosystems is to decrease the

target species and its impact on crops. For biological control to meet this goal, a multitrophic cascade of interactions from the biocontrol agent to the crop must occur (Begg et al., 2017; Morin, 2020), and this effect is not always observed (Begg et al., 2017; Letourneau et al., 2011). In this study, we observed that *P. punctiformis* infection was associated with a crop density increase in the reduced tillage treatment; after four years of patch infection crop densities equaled standard tillage levels. Even in higher density *C. arvense* stands, standard tillage may have disrupted the existing *C. arvense* enough to provide the crop an early competitive advantage, increasing germination, and seedling growth and recruitment. The four years of infection that it took for the crop stem density in the reduced tillage to equal standard tillage levels emphasizes the importance of patience when using biological control agents and underscores the difficulty with maintaining production in organic systems while transitioning to a reduced tillage integrated weed management plan. It also highlights the difference in time scales between biological control (long-term) and agricultural production (short-term), which is one of the major limiting factors in using biocontrol in agriculture (Charudattan & Dinoor, 2000).

Integrating non-inundative pathogenic biological control into agricultural weed management has received less research attention than other practices (Morin, 2020; Newman et al., 1998). Conservation biological control has largely focused on reducing/reversing the effects of agricultural intensification on natural enemies rather than actively manipulating the agroecosystem to promote their abundance (Begg et al., 2017). This study addressed these gaps in knowledge and demonstrated that *P. punctiformis* can be integrated with mechanical practices to manage *C. arvense* in semi-arid organic agroecosystems. However, to achieve sufficient control and increase annual cash crop production would likely take longer than the four years of the study. The observed increase of *P. punctiformis* symptoms, in response to reduced tillage, could help alleviate the challenges associated with access, inoculation, and scalability of *P. punctiformis* as a biocontrol agent. Producers and land managers who identify naturally occurring *P. punctiformis* infection could potentially expand the spread and impact of the biocontrol through long-term reduced-till management and reduce *C. arvense* over time.

CRediT authorship contribution statement

Christian Larson: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Daniel Chichinsky:** Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Funding acquisition. **Fabian Menalled:** Resources, Writing – review & editing, Supervision. **Tim Seipel:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2025.105724>.

References

- Bailey, K., Boyetchko, S., Derby, J., Hall, W., Sawchyn, K., Nelson, T., Johnson, D., & Spencer, N. (2000). Evaluation of fungal and bacterial agents for biological control of Canada thistle. Proceedings of the 10th International Symposium on Biological Control of Weeds, Bozeman, MT: Montana State University.
- Barbosa, P. A. (1998). Agroecosystems and conservation biological control. In P. A. Barbosa (Ed.), *Conservation biological control* (pp. 39–54). Academic Press.
- Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67 (1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Bean, D.W., Gladem, K., Rosen, K., Blake, A., Clark, R.E., Henderson, C., Kaltenbach, J., Price, J., Smallwood, E.L., Berner, D.K., 2024. Scaling use of the rust fungus *Puccinia punctiformis* for biological control of Canada thistle (*Cirsium arvense* (L.) Scop.): first report on a US statewide effort. *Biol. Control* 192, 105481.
- Begg, G.S., Cook, S.M., Dye, R., Ferrante, M., Franck, P., Lavigne, C., Lövei, G.L., Mansion-Vaquerie, A., Pell, J.K., Petit, S., 2017. A functional overview of conservation biological control. *Crop Prot.* 97, 145–158.
- Berner, D., Smallwood, E., Cavin, C., Lagopodi, A., Kashefi, J., Kolomiets, T., Pankratova, L., Mukhina, Z., Cripps, M., Bourdôt, G., 2013. Successful establishment of epiphytotes of *Puccinia punctiformis* for biological control of *Cirsium arvense*. *Biol. Control* 67 (3), 350–360.
- Berner, D., Smallwood, E., Cavin, C., McMahon, M., Thomas, K., Luster, D., Lagopodi, A., Kashefi, J., Mukhina, Z., Kolomiets, T., 2015. Asymptomatic systemic disease of Canada thistle (*Cirsium arvense*) caused by *Puccinia punctiformis* and changes in shoot density following inoculation. *Biol. Control* 86, 28–35.
- Bickler, A.J., Masiunas, J.B., 2009. Canada thistle (*Cirsium arvense*) suppression with buckwheat or sudangrass cover crops and mowing. *Weed Technol.* 23 (4), 556–563.
- Bockus, W., Shroyer, J., 1998. The impact of reduced tillage on soilborne plant pathogens. *Annu. Rev. Phytopathol.* 36 (1), 485–500.
- Bourdôt, G.W., Basse, B., Cripps, M.G., 2016. Mowing strategies for controlling *Cirsium arvense* in a permanent pasture in New Zealand compared using a matrix model. *Ecol. Evol.* 6 (9), 2968–2977.
- Bradshaw, M.J., Carey, J., Liu, M., Bartholomew, H.P., Jurick, W.M., Hambleton, S., Hendricks, D., Schnittler, M., Scholler, M., 2023. Genetic time traveling: sequencing old herbarium specimens, including the oldest herbarium specimen sequenced from kingdom Fungi, reveals the population structure of an agriculturally significant rust. *New Phytol.* 237 (4), 1463–1473.
- Burns, E.E., Prischmann-Voldseth, D.A., Gramig, G.G., 2013. Integrated management of Canada thistle (*Cirsium arvense*) with insect biological control and plant competition under variable soil nutrients. *Invasive Plant Sci. Manage.* 6 (4), 512–520.
- Charudattan, R., Dinoor, A., 2000. Biological control of weeds using plant pathogens: accomplishments and limitations. *Crop Prot.* 19 (8–10), 691–695.
- Chichinsky, D., Larson, C., Eberly, J., Menalled, F.D., Seipel, T., 2023. Impact of *Puccinia punctiformis* on *Cirsium arvense* performance in a simulated crop sequence [Original Research]. *Front. Agron.* 5. <https://doi.org/10.3389/fagro.2023.1201600>.
- Clark, A.L., Jahn, C.E., Norton, A.P., 2020. Initiating plant herbivory response increases impact of fungal pathogens on a clonal thistle. *Biol. Control* 143, 104207.
- Cripps, M.G., Gassmann, A., Fowler, S.V., Bourdôt, G.W., McClay, A.S., Edwards, G.R., 2011. Classical biological control of *Cirsium arvense*: lessons from the past. *Biol. Control* 57 (3), 165–174.
- Davis, S., Mangold, J., Menalled, F., Orloff, N., Miller, Z., Lehnhoff, E., 2018. A meta-analysis of Canada thistle (*Cirsium arvense*) management. *Weed Sci.* 66 (4), 548–557.
- Demers, A., Berner, D., Backman, P., 2006. Enhancing incidence of *Puccinia punctiformis*, through mowing, to improve management of Canada thistle (*Cirsium arvense*). *Biol. Control* 39 (3), 481–488.
- Eber, S., Brandl, R., 2003. Regional patch dynamics of *Cirsium arvense* and possible implications for plant-animal interactions. *J. Veg. Sci.* 14 (2), 259–266.
- Eilenberg, J., Hajek, A., Lomer, C., 2001. Suggestions for unifying the terminology in biological control. *BioControl* 46, 387–400.
- Esposito, M., Westbrook, A.S., Maggio, A., Cirillo, V., DiTommaso, A., 2023. Neutral weed communities: The intersection between crop productivity, biodiversity, and weed ecosystem services. *Weed Sci.* 71 (4), 301–311.
- Fox, J., & Weisberg, S. (2019). *An {R} companion to applied regression* (Third ed.). Sage publications. Thousand Oaks, CA, USA. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
- French, R., Lightfield, A., 1990. Induction of systemic aecial infection in Canada thistle (*Cirsium arvense*) by teliospores of *Puccinia punctiformis*. *Phytopathology* 80 (9), 872–877.
- Gontijo, L.M., 2019. Engineering natural enemy shelters to enhance conservation biological control in field crops. *Biol. Control* 130, 155–163.
- Graglia, E., Melander, B., Jensen, R., 2006. Mechanical and cultural strategies to control *Cirsium arvense* in organic arable cropping systems. *Weed Res.* 46 (4), 304–312.
- Gramig, G., Pull, Z.A., Carr, P.M., 2024. Diversified crop sequencing for suppression of creeping perennial weeds in organic production systems. *Farm. Syst.* 2 (1), 100070.

- Harker, K.N., O'Donovan, J.T., 2013. Recent weed control, weed management, and integrated weed management. *Weed Technol.* 27 (1), 1–11.
- Hettinger, K., Seipel, T., Miller, Z., Hubbel, K., 2023. Crop rotation and cultivation effects on *Convolvulus arvensis* population dynamics in small grain organic cropping systems. *Front. Agron.* 5, 1177461.
- Kennedy, A., Kremer, R., 1996. Microorganisms in weed control strategies. *J. Prod. Agric.* 9 (4), 480–485.
- Kentjens, W., Casonato, S., Kaiser, C., 2024. Californian thistle (*Cirsium arvense*): endophytes and *Puccinia punctiformis*. *Pest Manag. Sci.* 80 (1), 115–121.
- Kluth, S., Krues, A., Tschamtk, T., 2003. Influence of mechanical cutting and pathogen application on the performance and nutrient storage of *Cirsium arvense*. *J. Appl. Ecol.* 40 (2), 334–343.
- Landis, D., Menalled, F., Lee, J., Carmona, D., Perez-Valdez, A., 2000. Habitat management to enhance biological control in IPM. Emerging technologies for integrated pest management: concepts, research, and implementation. APS Press, pp. 226–239.
- Larson, C.D., Menalled, F.D., Lehnhoff, E.A., Seipel, T., 2021. Plant community responses to integrating livestock into a reduced-till organic cropping system. *Ecosphere* 12 (3), e03412.
- Larson, C.D., Hettinger, K., Carr, P.M., Miller, P.R., Volkman, M., Chichinsky, D., Seipel, T., 2024. Tillage and crop sequences for organic *Cirsium arvense* management in the Northern Great Plains. *Agron. J.* <https://doi.org/10.1002/agj2.21624>.
- Lehnhoff, E., Miller, Z., Miller, P., Johnson, S., Scott, T., Hatfield, P., Menalled, F.D., 2017. Organic agriculture and the quest for the holy grail in water-limited ecosystems: managing weeds and reducing tillage intensity. *Agriculture* 7 (4), 33. <https://doi.org/10.3390/agriculture7040033>.
- Lenth, R. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. In R package version 1.8.8. {<https://CRAN.R-project.org/package=emmeans>}.
- Letourneau, D.K., 1998. Conservation biology: lessons for conserving natural enemies. In: Barbosa, P.A. (Ed.), *Conservation Biological Control*. Academic Press, pp. 9–38.
- Letourneau, D.K., Ambrecht, I., Rivera, B.S., Lerma, J.M., Carmona, E.J., Daza, M.C., Escobar, S., Galindo, V., Gutiérrez, C., López, S.D., 2011. Does plant diversity benefit agroecosystems? a synthetic review. *Ecol. Applic.* 21 (1), 9–21.
- Liebman, M., Gallandt, E.R., Jackson, L., 1997. Many little hammers: ecological management of crop-weed interactions. *Ecol. Agric.* 1, 291–343.
- Lukashyk, P., Berg, M., Köpke, U., 2008. Strategies to control Canada thistle (*Cirsium arvense*) under organic farming conditions. *Renewable Agric. Food Syst.* 23 (1), 13–18.
- Mohler, C. L. (2001). Weed life history: identifying vulnerabilities. In M. Liebman, C. L. Mohler, & C. Staver (Eds.), *Ecological management of agricultural weeds* (pp. 40–98). Cambridge.
- Morin, L., 2020. Progress in biological control of weeds with plant pathogens. *Annu. Rev. Phytopathol.* 58, 201–223.
- Müller, E., Nentwig, W., 2011. Plant pathogens as biocontrol agents of *Cirsium arvense*—an overestimated approach? *NeoBiota* 11, 1–24.
- Newman, R. M., Thompson, D. C., & Richman, D. B. (1998). Conservation strategies for the biological control of weeds. In P. A. Barbosa (Ed.), *Conservation biological control* (pp. 371–396). Academic Press.
- Orloff, N., Mangold, J., Miller, Z., Menalled, F., 2018. A meta-analysis of field bindweed (*Convolvulus arvensis* L.) and Canada thistle (*Cirsium arvense* L.) management in organic agricultural systems. *Agr. Ecosyst. Environ.* 254, 264–272.
- Orr, D., & Fox, A. (2012). Augmentation and conservation of natural enemies. In D. Abrol & U. Shanker (Eds.), *Integrated pest management: principles and practice* (pp. 58–91). CAB.
- Pell, J., Hannam, J., Steinkraus, D., 2010. Conservation biological control using fungal entomopathogens. *BioControl* 55, 187–198.
- PRISM Climate Group. (2023). Time series values for individual locations. Date Accessed: 4 Jan 2024. <https://prism.oregonstate.edu/explorer/>.
- R Core Team, 2024. *R: A language and environment for statistical computing*. In R Foundation for Statistical Computing.
- Rajanna, G., Dass, A., Suman, A., Babu, S., Venkatesh, P., Singh, V., Upadhyay, P.K., Sudhishri, S., 2022. Co-implementation of tillage, irrigation, and fertilizers in soybean: impact on crop productivity, soil moisture, and soil microbial dynamics. *Field Crop Res.* 288, 108672.
- Rawat, L., Bisht, T., & Naithani, D. C. (2021). Plant disease management in organic farming system: strategies and challenges. In K. Sing, S. Jahagirdar, & B. Sarma (Eds.), *Emerging trends in plant pathology* (pp. 611–642). Springer. https://doi.org/10.1007/978-981-15-6275-4_27.
- Sciegienka, J.K., Keren, E.N., Menalled, F.D., 2011. Interactions between two biological control agents and an herbicide for Canada thistle (*Cirsium arvense*) suppression. *Invasive Plant Sci. Manage.* 4 (1), 151–158.
- Soil Survey Staff (NRCS). (2023). Web Soil Survey. Natural Resources Conservation Service, United States Department of Agriculture. Date Accessed: 3 April 2023. Retrieved from <http://websoilsurvey.sc.egov.usda.gov/>.
- Stenberg, J.A., Sundh, I., Becher, P.G., Björkman, C., Dubey, M., Egan, P.A., Friberg, H., Gil, J.F., Jensen, D.F., Jonsson, M., 2021. When is it biological control? a framework of definitions, mechanisms, and classifications. *J. Pest. Sci.* 94 (3), 665–676.
- Tautges, N.E., Goldberger, J.R., Burke, I.C., 2016. A survey of weed management in organic small grains and forage systems in the northwest United States. *Weed Sci.* 64 (3), 513–522.
- Termorshuizen, A. J. (2017). Ecology of fungal plant pathogens. *Microbiology Spectrum*, 4 (6), 387–397. <https://doi.org/10.1128/microbiolspec.funk-0013-2016>.
- Tiley, G.E., 2010. Biological flora of the British Isles: *Cirsium arvense* (L.) Scop. *J. Ecol.* 98 (4), 938–983.
- Triplett Jr, G.B., Dick, W.A., 2008. No-tillage crop production: a revolution in agriculture! *Agron. J.* 100, S-153-S-165.
- Wang, H., Guo, Q., Li, X., Li, X., Yu, Z., Li, X., Yang, T., Su, Z., Zhang, H., Zhang, C., 2020. Effects of long-term no-tillage with different straw mulching frequencies on soil microbial community and the abundances of two soil-borne pathogens. *Appl. Soil Ecol.* 148, 103488.
- Watson, A., & Keogh, W. (1981). Mortality of Canada thistle due to *Puccinia punctiformis*. *Proceedings of the 5th International Symposium on Biological Control of Weeds* (325–332).
- Weigel, M.M., Andert, S., Gerowitz, B., 2023. Monitoring patch expansion amends to evaluate the effects of non-chemical control on the creeping perennial *Cirsium arvense* (L.) Scop. in a spring wheat crop. *Agronomy* 13 (6), 1474.
- Westerman, P.R., Liebman, M., Menalled, F.D., Heggstadler, A.H., Hartzler, R.G., Dixon, P.M., 2005. Are many little hammers effective? Velvetleaf (*Abutilon theophrasti*) population dynamics in two- and four-year crop rotation systems. *Weed Sci.* 53 (3), 382–392.