

Biotic and abiotic drivers of pathogen prevalence in a rust fungus with multiple plant hosts

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Abstract

Pathogens can have strong negative effects on individual fitness that scale up to influence population dynamics. Because of this, understanding the ecological contexts that shape pathogen transmission and prevalence is critical for understanding community dynamics and ecosystem function. *Puccinia monoica* is a fungal pathogen with a complex life cycle that alternates between infecting grasses and forbs in the Brassicaceae. This study investigates how *B. stricta* and grass host densities, as well as abiotic factors such as drought, influence transmission of *P. monoica*. I conducted field surveys near Gothic, Colorado, to quantify environmental conditions across multiple sites with variation in host and pathogen abundance. Furthermore, to experimentally test the potential role of drought in facilitating pathogen spread, I performed controlled inoculations of grass hosts under drought and control conditions in a greenhouse. These integrated field and experimental approaches helped reveal how spatial variation in biotic and abiotic environments shape pathogen dynamics in multispecies communities. By quantifying relationships between host density, environmental stress, and pathogen transmission, this study supports our understanding of the population ecology of plant-pathogen systems which could inform models of disease spread under changing climate conditions.

Introduction

Plant diseases have significant impacts on the community dynamics of natural systems (Seabloom et al., 2015). The transmission and severity of plant diseases can be influenced by the traits of the plant host(s), traits of the pathogen, and environmental conditions (Kumar & Mukhopadhyay, 2025). Patterns of pathogen spread can be particularly complex when transmission depends upon interactions in multi-pathogen and/or multi-host communities. Nevertheless, understanding these multi-pronged relationships is important for anticipating how climate change may affect ecosystems jointly by affecting species interactions, environmental tolerances, and disease susceptibility.

Biotic factors can influence infection dynamics through complex interactions between hosts, pathogens, and other organisms in their shared environments. For example, in systems harboring multiple coexisting pathogens, pathogen-pathogen interactions can lead to immunosuppression in the host, altering susceptibility (Seabloom et al., 2015). Inter-guild interactions can also shape dynamics, for example if insect visitation influences a pathogen's sexual reproduction or dispersal (Roy, 1993a). Pathogens that are obligate biotrophs, like rust fungi, rely on living host tissues to survive and reproduce, and often exhibit strong host specificity (Helfer 2014), making their transmission dynamics particularly sensitive to the structure and diversity of the surrounding plant community. Specifically, community composition can influence the availability of susceptible hosts, modify local microclimates, and/or mediate indirect interactions such as apparent competition or facilitation among host species. These community-level processes shape not only which hosts are available but also local pathogen pressure, ultimately influencing the prevalence and impact of pathogen dispersal and reproductive success (Roy et al., 1998).

Abiotic stress can also impact infection dynamics by altering host-pathogen interactions. Abiotic environmental stressors such as temperature, humidity, and drought can decrease host survivorship and increase host susceptibility (Vicente-Santos, 2023). Leaf surface moisture and relative humidity also strongly influence the establishment of fungal pathogens on host plants (Helfer, 2014). Drought effects on host plants have been shown to both exacerbate and mitigate disease outbreaks, depending on the plant pathogen system, the timing and intensity of drought, and organ infected (Choudhary & Senthil-Kumar, 2024). On the one hand, drought can enhance host resistance by inducing stomatal closure, epidermal thickening, and lignification, which impede pathogen entry into plant tissue. On the other hand, drought can also damage photosynthetic capacity and reduce plant defense expression, increasing susceptibility to certain pathogens. Drought can also directly influence pathogen survival, growth, and virulence outside the host, favoring desiccation-resistant pathogens or altering hyphal growth patterns to promote spread (Choudhary & Senthil-Kumar, 2024). Abiotic stress further shapes pathogen transmission dynamics by modifying the outcomes of competition and coexistence among pathogens within hosts, influencing which strains or species prevail under different environmental conditions. Thus, abiotic conditions interact with host and pathogen traits in context-dependent ways that can either facilitate or constrain pathogen spread.

While both biotic and abiotic factors have been shown to strongly influence plant-pathogen interactions, comparably few studies have explored their joint effects on

transmission and infection prevalence in wild populations. The rust fungus *Puccinia monoica* is an excellent study system for exploring how biotic contexts and abiotic stress interact because it has a complex life cycle involving alternation of hosts between co-occurring plants in different families (Roy, 1993a, 1993b). Past work focusing on infection within the mustard genus *Boechnera* has revealed that this pathogen-mediated floral mimicry is crucial for the fungus's sexual reproduction, as *P. monoica* requires insects to transfer spermatia (fungal gametes) between different mating types that form on pseudoflowers. The reproductive success of *P. monoica* is thus influenced by pollinator community dynamics and competition among co-flowering species (Roy et al., 1998). However, despite this pathogen requiring multiple plant host species to complete its life cycle, little is known about how plant community composition influences transmission. Furthermore, the consequences of abiotic stressors on *P. monoica* transmission—through direct effects on the fungal pathogen and/or effects on the host plants, which may differ across multiple host species—have not yet been explored.

In this study, I combined field surveys with an experimental manipulation of drought to answer three research questions: (1) How do biotic factors and abiotic environmental stressors jointly influence the prevalence of *Puccinia monoica* infection in *Boechnera stricta*? and (2) Does drought stress increase pathogen success in alternate grass hosts? I hypothesize that higher host densities and drought stress will increase pathogen prevalence.

Methods

Study system

Puccinia monoica is an obligate parasitic rust fungus with a complex life cycle involving multiple spore stages that infect plant hosts in both the mustard (Brassicaceae) and grass (Poaceae) families (**Figure 1**). In the mustard host genus *Boechnera*, *P. monoica* causes systemic infection that affects all future growth. In the late summer, wind-dispersed spores from grass hosts infect *B. stricta* rosettes, leading to systemic infections that reprogram the plant's meristems over winter. By spring, infected plants produce pseudoflowers instead of true flowers (**Figure 2**). These structures are bright yellow flower-like clusters of leaves that mimic co-occurring blooms such as *Ranunculus* spp. and are visually and chemically different from typical *Boechnera* host flowers (Roy, 1993; Cano et al., 2013). Pseudoflowers are covered with a sticky, sweet-smelling exudate attractive to insects like bees, butterflies, and flies. Pseudoflower development following *P. monoica* infection involves transcriptional

changes in host genes related to shoot and leaf development, volatile organic compound synthesis, and sugar metabolism (Cano et al., 2013). As a result of this developmental hijacking to promote fungal rather than plant reproduction, *P. monoica* infection increases host mortality and almost always eliminates host reproduction (Roy, 1993a).

P. monoica relies on insect visitation to transport spermatia spores of different mating types among pseudoflowers (Roy, 1993a; Roy & Raguso, 1997). When two compatible spermatia (type + or -) fuse and sexually recombine, *P. monoica* then begins making aeciaspores which are wind-dispersed from the mustard host onto a grass host. Once the grass host is infected the pathogen can keep infecting other grasses throughout the growing season (Roy, 1993b). Finally, when the grass senesces, basidiospores are wind-dispersed onto mustard rosettes, allowing the life cycle to repeat (Roy, 1993a). Importantly, completion of the fungal life cycle requires presence and successful infection of both alternate hosts (**Figure 1**), and the aeciaspores produced on mustard pseudoflowers are only infectious to grasses (i.e., mustards do not spread infection directly to one another). This fungal life history allows the grass host to be continuously infected through the growing season, but the mustard host can only be infected once per season (Roy, 1993).

While many species in many genera of the Brassicaceae can become infected by *Puccinia* (Roy, 1993a), *P. monoica* pseudoflowers near Gothic, CO are frequently observed on individuals in the genus *Boechera*, including *B. retrofracta* (formerly *Arabis holboellii*) and *B. stricta* (formerly *Arabis drummondii*). The *Boechera* genus is a well-studied model for ecological and evolutionary research, due to its self-compatibility, widespread distribution, and ecological variation (Rushworth et al., 2022). The grass species that are alternate *P. monoica* hosts are *Trisetum spicatum*, *Koeleria cristata*, *Stipa elmeri* and *Stipa occidentalis* (Cummins, 1962).

Field survey

I selected 6 of sites near Gothic, CO varying in infection prevalence (i.e., proportion of *Boechera* hosts showing pseudoflower formation). In each site I established several 20 m transects that spanned a representative area of the population. The total number of transects per site ranged from 4 to 16, depending on the area of suitable *Boechera* habitat at each study site (Table 1). I estimated infection prevalence by counting the number of uninfected (typical morphology) and infected (pseudoflower) *B. stricta* within 1 meter of either side of each transect. I calculated infection prevalence per transect by dividing the amount of infected plants by the total

amount of infected and uninfected plants. Site-average infection prevalence ranged from 0 to 21% (**Table 1**).

To understand the effects of *B. stricta* infection by the proximity to the alternate grass host, I randomly selected 5 pseudoflowers and 5 uninfected *B. stricta* at each site and recorded the distance of the closest 10 *T. spicatum* individuals within a 10m radius from the target plant. If sites had low infection prevalence, I measured *T. spicatum* distances from 5 uninfected *B. stricta* and 0-5 pseudoflowers, depending on availability. This was done to compare distances to *T. spicatum* from both infected and uninfected *B. stricta*, and test whether spatial host arrangement influences the probability of infection. To assess the effects of soil properties on infection, I collected 100 grams of soil from each site for laboratory analysis of soil texture and water holding capacity.

Environmental data

Soil field capacity refers to the amount of water soil can retain after excess water has drained away, it is the water available to plants for uptake. After sieving soil to 2 mm particle size, I measured the field capacity using a 10 g subset from each soil sample. I weighed the soil that was set aside, tared a beaker containing a funnel with a wet filter, then added the soil sample and water until it stopped dripping. I then recorded the mass of the saturated soil, placed the samples in an oven for 24 hours and recorded the dry weight. I estimated field capacity as follows:

$$\text{Field capacity} = \frac{\text{mass of saturated soil} - \text{mass of dried soil}}{\text{mass of dried soil}}$$

Soil texture refers to the relative proportions of sand, silt, and clay particles in a soil. It influences pore size distribution, water retention, drainage, aeration, and root penetration— factors which may have direct and indirect effects on plant–pathogen interactions. I estimated soil texture using the remaining soil sample from each site. To do so, I filled mason jars 1/3 with sieved soil and up to 2/3 with soapy water. I shook it for 30 seconds and let it settle to separate the sand, silt and clay and analyze their percent composition. Specifically, I measured the layer of sediment in the jar at 40 seconds for sand, 2 hours for silt, and 24 hours for clay compositions. I then subtracted the height of each layer from the ones below them and divided the size of each individual layer by the total height of all layers to calculate percentage composition (Andrew Jeffers, n.d.). I used a soil texture triangle to determine the soil texture and soil classification from each sample.

I acquired Wetness Index (1-m resolution topographic index estimating relative soil moisture based on landscape position and height above stream channels) and NDVI (Normalized Difference Vegetation Index, derived from satellite or aerial imagery to estimate vegetation greenness and photosynthetic activity) data from the RMBL Spatial Data Platform based on GPS locations collected using a Trimble GPS unit. Climate data was obtained from gridded projections at spatial resolutions of 1 meter. I selected these variables because they provide complementary measures of environmental variation that can influence *P. monoica* infection dynamics. The Wetness Index estimates relative soil moisture potential from topographic position, offering a fine-scale proxy for microclimatic moisture conditions that can affect spore germination, survival, and host plant physiology. NDVI, derived from remotely sensed vegetation reflectance, serves as an index of vegetation greenness and productivity, which can reflect habitat quality and host plant vigor. Together, these metrics may relate to infection patterns via spatial variation in both moisture availability and vegetative cover.

Inoculation experiment

I complemented my field survey with an experiment manipulating drought to determine whether abiotic stress alters *P. monoica* transmission by changing host plant susceptibility. I selected the grass host to be the target species for this experiment due to its localized infection, the ability to measure the infection within a growing season, and the ease of identifying infected *B. stricta*, which produce spores infectious only to grasses (**Figure 1**). My field survey revealed that *Trisetum spicatum* was the most abundant alternate host co-occurring with *Boechera* in my six study sites. Thus, I used *T. spicatum* as the focal species in my inoculation experiment. I dug up 6 bunches of *T. spicatum* from each of 3 field sites (N=18), transplanted them into pots, and then maintained them indoors under grow lights. When collecting grasses in the field, I visually screened for signs of fungal pathogens (e.g. blight, leaf spots, rotting, rusts, etc.) and avoided collecting grasses that were already infected. I also selected plants that appeared to be at similar phenological stages, and small enough to fit under grow lights.

After transplanting, I assigned the pots to one of two controlled watering treatments: well-watered (watered to saturation at 48 hour intervals) and drought treatment (withholding water for the duration of the experiment). I measured soil moisture in each pot at the end of the experiment using a handheld probe (Fieldscout TDR 150) to assess the efficacy of the watering treatment 5 days after initiating the watering treatment. The watering manipulation significantly reduced volumetric water

content in pots assigned to the drought treatment ($p = <0.0001$; **Supplementary Figure 1**).

I collected grass-infecting aeciaspores from an infected *B. stricta* by harvesting a pseudoflower at the closest documented infection location to Gothic Townsite (Ross Meadow) and agitating leaf samples with aecia (spore-producing structures) in distilled water. I then experimentally inoculated potted grass hosts in both watering treatments with aeciaspores using a spray bottle containing the spore suspension. I sprayed approximately 1.5mL of the suspension onto each plant. Using a dissecting microscope to count spores in a 1 mL subsample, I estimated the concentration of spores to be 140 spores/mL. Thus, each grass was inoculated with approximately 210 spores. Five days following inoculation, I screened for infection by counting the number of infection sites within a 100cm leaf sample using a dissecting microscope. I estimated “infection density” as the number of sites/100cm.

Data analysis

I analyzed the data collected from both the field surveys and inoculation experiment to test which biotic and abiotic factors influence the prevalence and transmission of *P. monoica*. For data from my field survey, I used linear regressions to analyze the transect level and site level predicted drivers of infection. At the transect level, I used multiple linear regression to test whether infection prevalence varied in response to Wetness Index, NDVI, *B. stricta* density, site, and all two- and three-way interactions. I also tested whether infected plants were closer to alternate grass hosts than were uninfected plants using an ANOVA including infection status (infected vs. uninfected), site, and their interaction as independent factors.

At the site level, I analyzed infection prevalence in response to the following abiotic variables: mean wetness, mean field capacity, mean sand and mean clay compositions. (I excluded the effect of mean proportion of silt as a descriptor of soil texture because it was strongly negatively correlated with mean proportion of sand.) I fit a separate model analyzing infection prevalence in response to biotic factors: *B. stricta* density, mean distance to *T. spicatum* alternate hosts, and NDVI. Due to limited sample size at the site level ($N=6$), I was unable to combine biotic and abiotic variables and interactions into a single statistical model.

Finally, for data from my lab inoculation experiment, I conducted an ANOVA comparing infection density between two watering treatments (well-watered vs. drought) in potted *T. spicatum* individuals.

Results

To evaluate drivers of *Puccinia monoica* infection in *Boechera stricta*, I analyzed data from both field surveys and a controlled inoculation experiment. These two complementary approaches allowed me to assess how biotic and abiotic variables influence infection prevalence in natural and experimental conditions.

At the transect level, both biotic and abiotic factors were correlated with *P. monoica* infection prevalence (**Table 2**). Infection prevalence was positively correlated with *B. stricta* density (**Figure 3**, $p=0.035$). Of all transect-level abiotic factors considered, the Wetness Index was the only significant predictor of infection; while there was no main effect of wetness, it interacted significantly with host density to predict infection prevalence (**Figure 4**; $p = 0.0151$). NDVI was not a significant predictor of infection. Pairwise distance measurements revealed that infected *B. stricta* plants were also significantly closer to the nearest alternate grass host compared to uninfected individuals (**Table 3**; **Figure 5**; $p = 0.0076$).

At the site level, infection prevalence showed a marginally significant negative correlation with field capacity (**Figure 7**; $p = 0.0739$). However, site-level infection prevalence was not correlated with NDVI, *B. stricta* density, mean distance between *B. stricta* and *T. spicatum*, wetness index, or soil texture (**Tables 4-5**).

In my inoculation experiment isolating the effect of drought stress on infection in grass hosts, grasses grown under drought conditions exhibited significantly lower infection densities compared to well-watered controls (**Figure 7**; $p = 0.04676$).

Discussion

This study integrated field observations and experimental data to understand the biotic and abiotic drivers of *Puccinia monoica* infection dynamics. My findings support the hypothesis that biotic interactions drive *P. monoica* infection at fine spatial scales. The positive association between *B. stricta* density and infection prevalence (**Figure 4**) suggests that higher host availability likely promotes pathogen transmission. Similarly, infected *B. stricta* individuals were, on average, significantly closer than uninfected *B. stricta* to *T. spicatum* alternate hosts (**Figure 6**). This suggests that host proximity or, more generally, spatial distributions of multiple species in communities, likely contribute to transmission dynamics in multi-host pathogens with obligate alternation. Greater local densities of *B. stricta* may increase the likelihood that wind-dispersed basidiospores land on susceptible individuals, while nearby grass hosts may facilitate reciprocal infection with aeciospores. These results are consistent with

earlier work on *P. monoica* infections of *Boechera* (Roy 1993b), other rust fungi (Helfer 2014), and more general models of pathogen transmission (Seabloom et al. 2015), all of which emphasize the role of community composition, host density, and spatial configurations in shaping pathogen prevalence. The significant interaction between transect-level Wetness Index and host density suggests that infection is most likely to occur where both host density and favorable microclimate coincide. Specifically, positive relationships between wetness and infection prevalence become stronger as *B. stricta* host densities increase (**Figure 5**). This may be because moist microenvironments enhance spore germination or increase host susceptibility. Laboratory manipulations of drought supported these results: *T. spicatum* grasses in the drought treatment were less susceptible to infection, showing development of fewer infection sites following inoculation. This implies that wet conditions not only influence fungal viability but also may interact with host physiology in ways that promote infection, likely by increasing leaf turgor or weakening physical barriers to entry (Choudhary & Senthil-Kumar, 2024).

While fine-scale analyses focusing at transect-level patterns within sites identified strong relationships between host density and spatial distributions, microclimate, and infection prevalence, broader-scale analyses did not uphold these patterns. At the site level, infection prevalence was not correlated with *B. stricta* density, average distance to alternate grass hosts, NDVI, or wetness. A marginally significant negative relationship between infection prevalence and field capacity (the amount of water soil can retain after excess has drained away) opposed findings at the transect level, with sites with soils that hold less water showing higher average rates of infection (**Figure 7**). This opposite pattern may have arisen because field capacity may reflect broad hydrological conditions (e.g. seasonal snowmelt infiltration patterns or sub-surface water storage) that are less relevant to short term, leaf-surface moisture conditions influencing spore germination or infection establishment at microsites. Soil water retention at larger scales could also influence pathogen infection rates by altering long term host vigor, competitive dynamics among plant species, or the persistence of pathogen propagules in the soil environment, potentially affecting background inoculation levels. These broader scale relationships may therefore be shaped by multiple interacting factors not captured in this study.

While this study reveals interesting patterns describing *P. monoica* infection prevalence, future work could strengthen these findings. The limited number of study sites I surveyed (n = 6) yielded limited statistical power to detect site-level patterns and limits generalizability. Spatial environmental covariates (NDVI and wetness index) derived from remote sensing may not accurately capture microsite conditions relevant to

pathogen biology. In the inoculation experiment, infection pressure was not standardized via spore counts, and longer-term effects of disease intensity or delayed symptom development were not assessed due to time constraints. Future research should increase site replication and compare multi-year disease dynamics. Molecular analyses of fungal gene expression under different moisture regimes may also reveal how abiotic stress influences pathogen development. Combining experimental and observational approaches will be critical for predicting disease spread under future climatic conditions.

In conclusion, this study shows that both biotic and abiotic factors influence the spatial patterns of *P. monoica* infection in wild populations of *B. stricta*. *B. stricta* host density and proximity to *T. spicatum* alternate hosts are strongly positively correlated with infection at the microsite scale, and these effects are enhanced under wet microclimatic conditions. Site-level variation in infection was less predictable but may relate to complex interactions between field capacity and host-pathogen dynamics. The results of the inoculation experiment support microsite-scale field findings, showing that drought can suppress fungal success in grasses. As climate change intensifies drought conditions, understanding these mechanisms becomes essential for forecasting pathogen impacts on natural plant communities.

Figures

Figure 1: Comparison of an uninfected *B. stricta* (left) and an infected *B. stricta* with pseudoflowers (right).



Figure 2: Lifecycle of *Puccinia monoica*, showing alternation between mustard and grass hosts (adapted from Roy, 1993).

A. Puccinia monoica Arth.

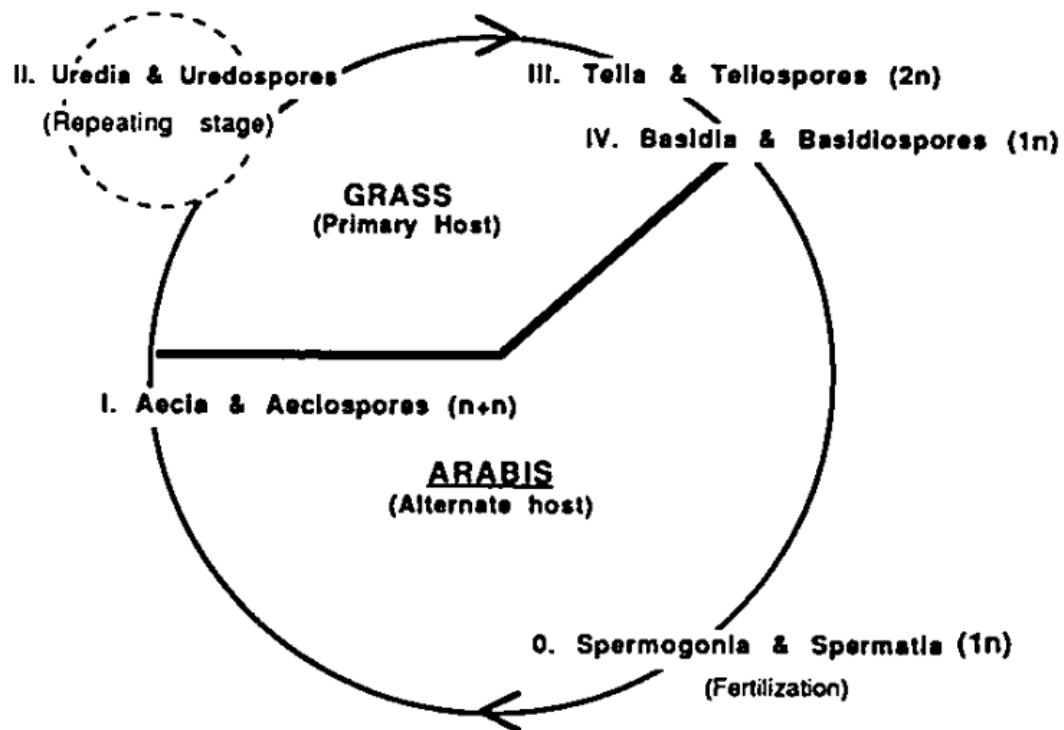


Figure 3: Map of the six study sites near Gothic, Colorado, where *Boechera stricta* populations were surveyed for *Puccinia monoica* infection in 2025. Sites include Avery, Cement Creek, Emerald Lake, Ross Meadow, Rustlers Gulch, and Stonefield.

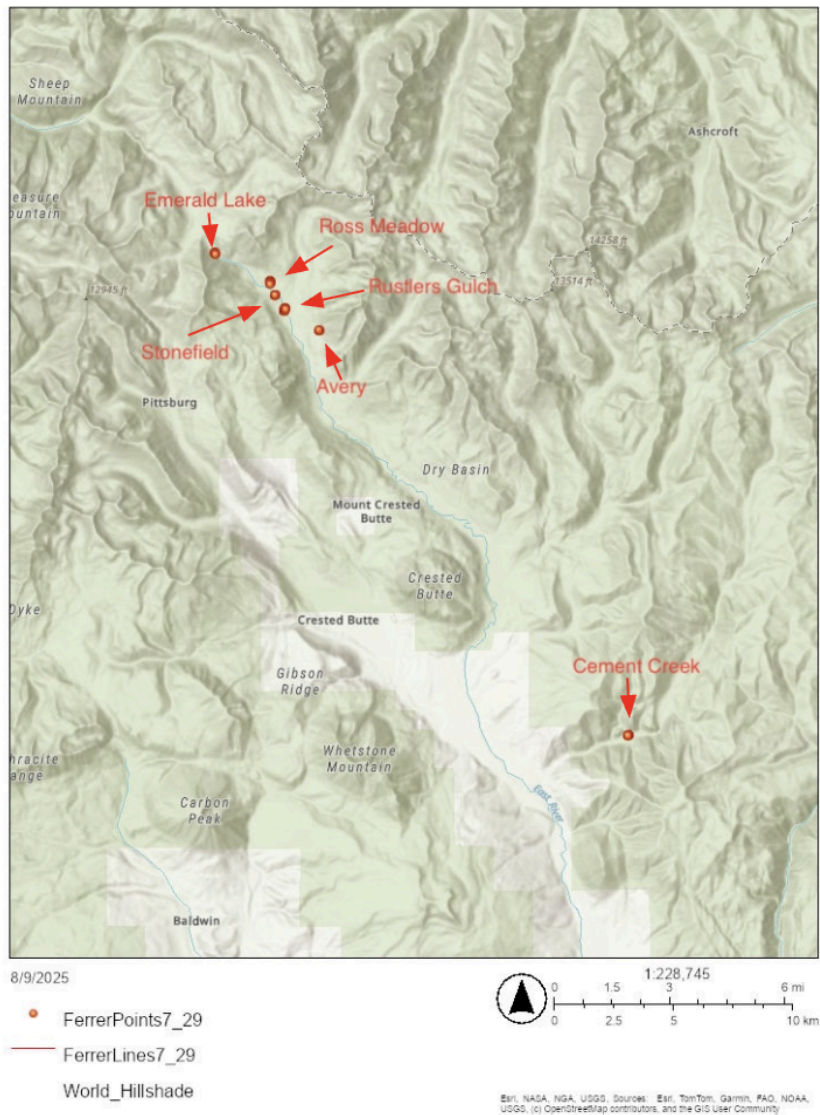


Table 1. Summary of *Boechera stricta* density and *Puccinia monoica* infection prevalence across six study sites near Gothic, Colorado. Values are means $\pm$ standard deviation. Number of 20 m transects surveyed at each site is shown in the second column.

Site	Transects	Mean <i>B. stricta</i> density (+/- SD)	Mean infection prevalence (+/- SD)
Avery	4	0.51 $\pm$ 0.45	0 $\pm$ 0
Cement Creek	4	0.03 $\pm$ 0.01	0 $\pm$ 0
Emerald Lake	8	0.31 $\pm$ 0.18	0.21 $\pm$ 0.19
Ross Meadow	10	0.62 $\pm$ 0.56	0.12 $\pm$ 0.24
Rustlers Gulch	6	0.39 $\pm$ 0.24	0.2 $\pm$ 0.22
Stonefield	16	0.73 $\pm$ 0.34	0.14 $\pm$ 0.13

Table 2: Results of multiple linear regression testing the effects of Wetness Index, NDVI, *B. stricta* density, site, and their interactions on *P. monoica* infection prevalence at the transect level. Significant predictors ($p < 0.05$) are in bold; marginal effects ($p < 0.1$) are indicated with “.”.

Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
wet	1	0.02779	0.027786	1.2896	0.26384
ndvi	1	0.0716	0.0716	3.323	0.07687 .
B. density	1	0.15774	0.15774	7.3209	0.01046
Site	5	0.29327	0.058654	2.7222	0.03522
wet:ndvi	1	0.00868	0.008681	0.4029	0.52974
wet:B. density	1	0.15625	0.156251	7.2518	0.0108

ndvi:B. density	1	0.01149	0.011488	0.5332	0.47013
wet:ndvi:B. density	1	0.02035	0.020352	0.9446	0.33778
Residuals	35	0.75413	0.021547	NA	NA

Table 3: Results of ANOVA testing the effects of infection status (infected vs. uninfected), site, and their interaction on the mean distance from *B. stricta* individuals to the nearest *T. spicatum* plants. Significant effects ($p < 0.05$) are in bold.

Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Infection	1	1929934	1929934	40.8358	5.45E-10
Site	4	369513	92378	1.9546	0.10107
Infection:Site	3	573066	191022	4.0419	0.00762
Residuals	340	16068683	47261	NA	NA

Table 4: Results of univariate regressions testing the effects of *B. stricta* density, mean distance to *T. spicatum*, and mean NDVI on *P. monoica* infection prevalence at the site level ($n = 6$ sites). No predictors were statistically significant at $p < 0.05$

Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
B. density	1	0.0055226	0.0055226	2.5322	0.3572
mean_distance	1	0.0202056	0.0202056	9.2647	0.2021
mean_ndvi	1	0.0005833	0.0005833	0.2675	0.6962
Residuals	1	0.0021809	0.0021809	NA	NA

Table 5: Results of univariate regressions testing the effects of abiotic variables (mean wetness, mean field capacity, mean sand %, mean clay %) on *P. monoica* infection prevalence at the site level (n = 6 sites). Marginal significance ($p < 0.1$) is indicated with “.”.

Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
mean_wet	1	0.0130949	0.0130949	32.8381	0.11
mean_fc	1	0.0293283	0.0293283	73.5465	0.0739 .
mean_sand	1	0.0000037	0.0000037	0.0094	0.9386
mean_clay	1	0.0008382	0.0008382	2.102	0.3844
Residuals	1	0.0003988	0.0003988	NA	NA

Table 6: Results of ANOVA testing the effect of watering treatment (well-watered vs. drought) on infection density (number of infection sites per 100 cm leaf) in *T. spicatum* during the inoculation experiment. Significant effects ($p < 0.05$) are in bold.

Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	180.5	180.5	4.6431	0.04676
Residuals	16	622	38.875	NA	NA

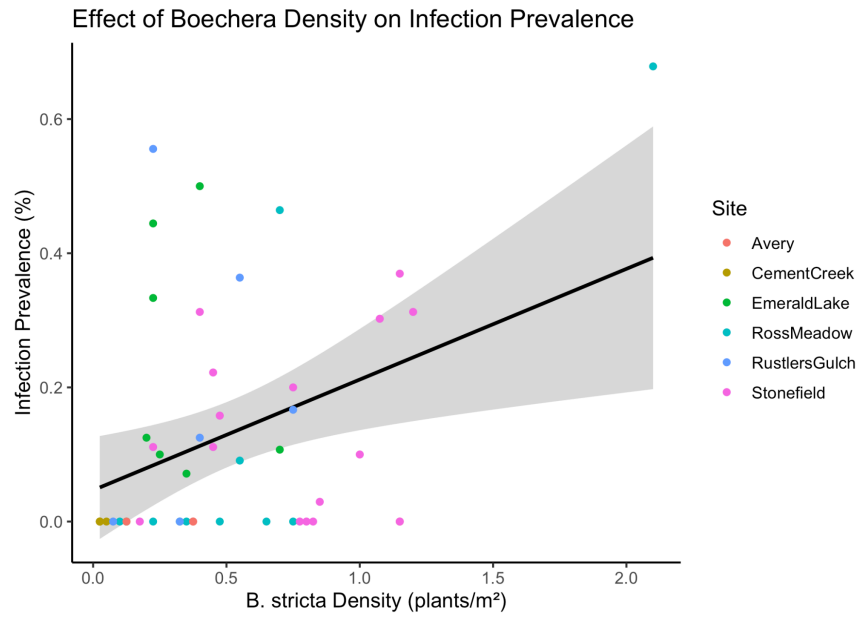


Figure 4: Relationship between *B. stricta* density and infection prevalence at the transect level.

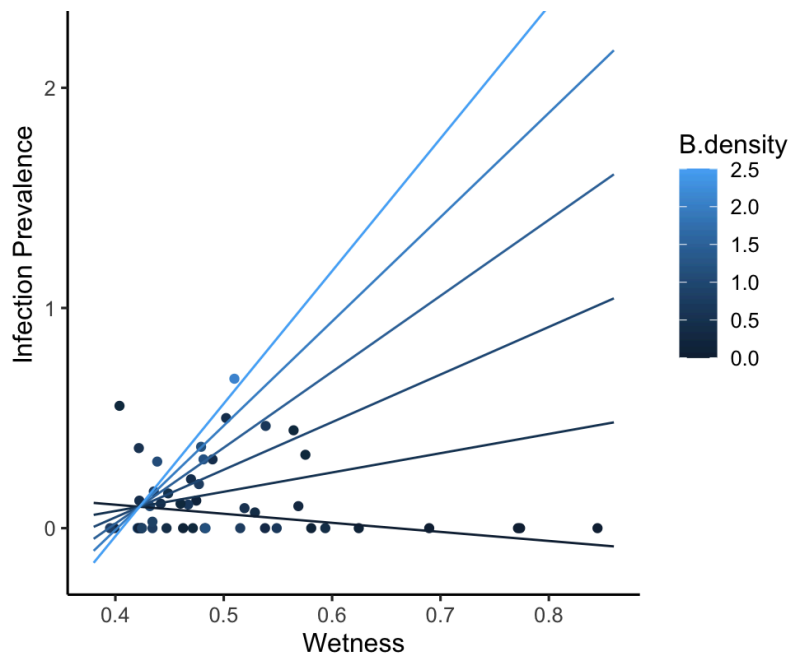


Figure 5: Interaction between Wetness Index and *B. stricta* density predicting infection prevalence at the transect level.

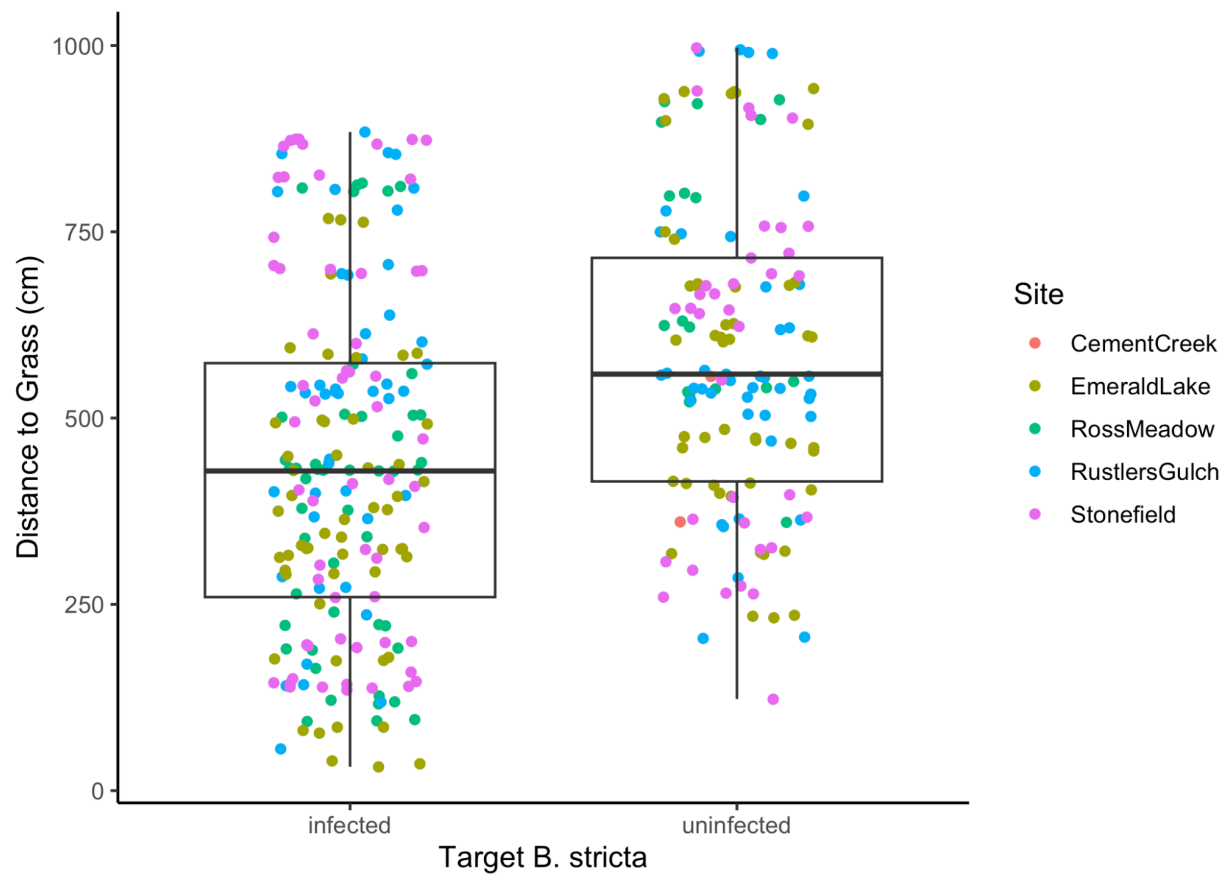


Figure 6: Mean distance to *T. spicatum* for infected vs. uninfected *B. stricta*.

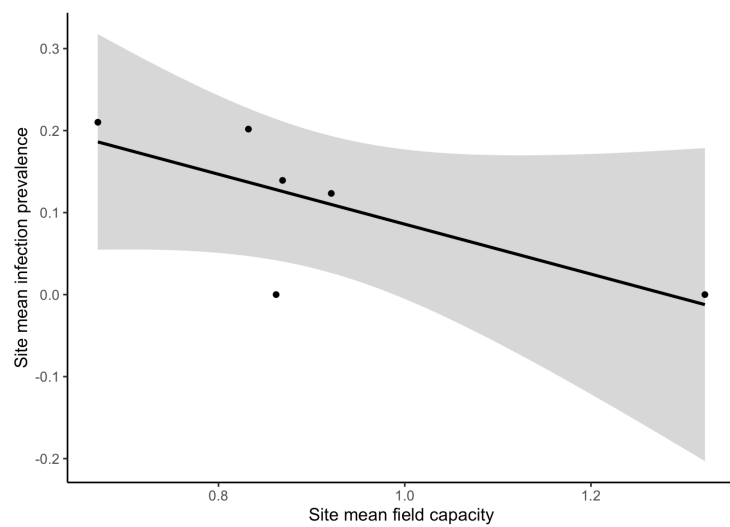


Figure 7: Relationship between site-level field capacity and infection prevalence.

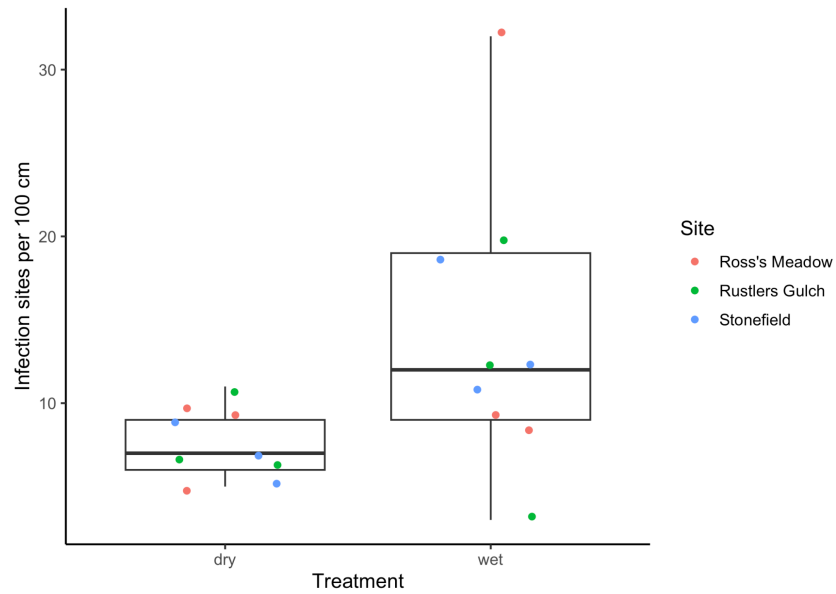
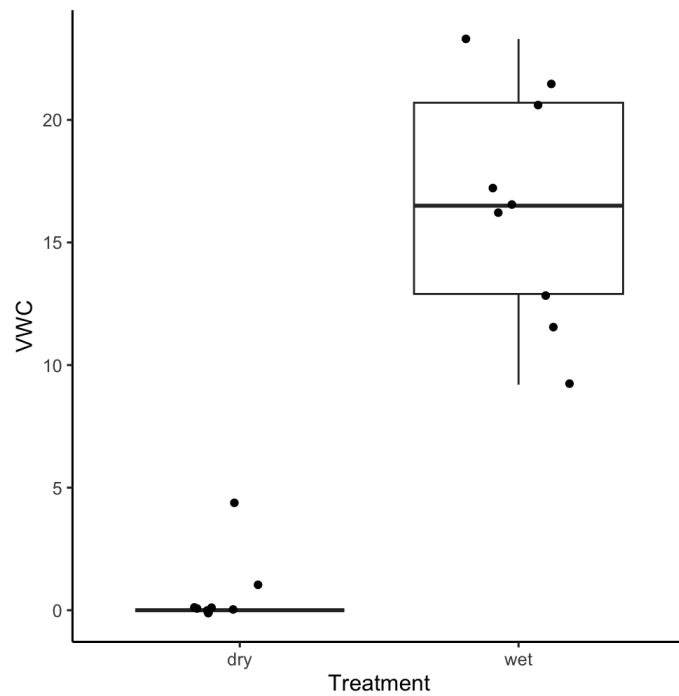
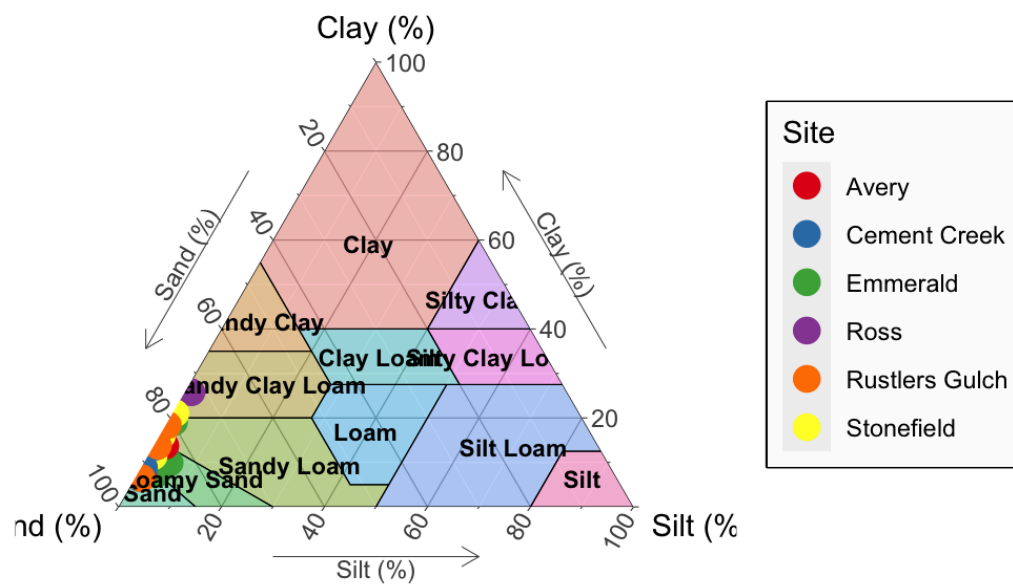


Figure 8: Mean infection density in *T. spicatum* under well-watered and drought treatments.

Supplementary figures



Supplementary Figure 1: Validation of drought treatment effectiveness in the inoculation experiment.



Supplementary Figure 2: Soil texture triangle showing percent sand, silt, and clay for each study site. Points represent site level mean values, with USDA soil texture classes indicated by boundaries. The plot illustrates differences in soil classification among sites, which may influence water retention and, indirectly, *P. monoica* infection dynamics.

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