

PREVALENCE AND SEVERITY OF *Melampspora lini* ON *Linum lewisii* UNDER EARLY SNOWMELT CONDITIONS

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ABSTRACT:

Plant-pathogen interactions act as a selective pressure on both parties involved. Changes in their environment can cause these relationships to shift in favor of either party. Subalpine ecosystems are experiencing increased drought, early snowmelt, and advancing phenology as a result of climate change. Snowmelt typically signals key life cycle events for subalpine plants. *Linum lewisii* is a perennial subalpine plant that is experiencing changes in phenology as snowmelt dates shift. *Melampspora lini* is a fungal parasite that relies on *Linum lewisii* for its entire life cycle. As the phenology of *L. lewisii* changes with early snowmelt, is it unclear how prevalence and severity of *M. lini* will change. There is the potential for negative impacts on both parties, so comparing infection to demographic metrics like size and reproductive success can help us understand how climate change will affect subalpine plants; both directly through life cycle changes, and indirectly through changing plant-pathogen dynamics. Following 430 individuals, we analysed the variation in prevalence, severity, and reinfection across treatment, and the variation in demography with infection status. Size was found to be the largest determinant of infection status, so we controlled for it as a covariate in all analyses. Broadly we found a higher prevalence of rust in control treatments, and we found that infected plants were more likely to flower. Both of these factors can shift community demographics in unexpected ways, which will likely occur more frequently as climate change progresses.

INTRODUCTION:

The interactions between a parasite and its plant host, otherwise collectively known as a pathosystem, play a critical role in shaping ecosystem structures through coevolution (Duggal *et al.* 2023; Höckerstedt *et al.* 2022). This pressure can influence the species and genetic diversity of plants within an ecosystem, as well as the fitness of individuals (Gilbert 2002). Pathogens are often constrained by environmental, geographic, and ecological factors (Anderson *et al.* 2004; Bebbler *et al.* 2013; Duggal *et al.* 2023). When there is a shift in any of these factors, which act as boundaries for the spread of infection, there is the potential for diseases to get a leg up on their hosts (Bebbler *et al.* 2013; Jiranek *et al.* n.d.; Miller *et al.* 2022; Velásquez *et al.* 2018).

One aspect within subalpine systems that is changing due to climate change is snowmelt. The timing of snowmelt has been a key determining environmental factor for plant phenology over the past few decades (Iler *et al.* 2013; Inouye 2008; Rose-Person *et al.* 2024; “Snow Drought Current Conditions and Impacts in the West | May 1, 2025 | Drought.gov” 2025). Climate change can vary in severity across ecosystems, but also vary in severity among different climate variables (Forrest & Miller-Rushing 2010). Even if a species can maintain its climatic niche throughout shifts in temperature and snowmelt, it is not guaranteed that they are able to adapt to the altered abiotic factors present within the ecosystem (Iler *et al.* 2021) – or

compete with the adaptations of other species.

Snowmelt is a vital signal in a plant's life cycle (Rose-Person *et al.* 2024; Stanton *et al.* 1994). During the winter, snow provides protection from freezing temperatures and insects by acting as an insulator (Inouye & McGuire 1991). In spring, snow melt provides increased soil moisture for perennial plants, which helps them prepare for late spring and summer (McNamara *et al.* 2005; Stanton *et al.* 1994). Additionally, exposure to sun and a warming environment after snowmelt signal processes within plants to start producing above ground vegetation (Inouye & McGuire 1991; Jonas *et al.* 2008; Rose-Person *et al.* 2024). After investing in above ground vegetation, plant growth plateaus as the individual shifts to primarily investing in reproductive structures (Iler *et al.* 2021; Totland & Alatalo 2002). The implications of a longer growing window for flowering plants in sub-alpine ecosystems is still being investigated, as abiotic and biotic factors shift alongside plant communities (Iler *et al.* 2021).

As early snowmelt changes the cycling of plant processes, the life cycles of obligate parasites in plant populations also begins to change (Duggal *et al.* 2023; Rose-Person *et al.* 2024). The range of an obligate parasite must overlap completely with its host, and is highly dependent on climatic and weather conditions due to narrow tolerances for variation in environment (van den Berg & van den Bosch 2007; Duggal *et al.* 2023; Shaw & Osborne 2011; Velásquez *et al.* 2018). Changes in precipitation, temperature, community composition, and host phenology has the potential to shift the overlap and alter the ways in which parasites exist within their host (Anderson *et al.* 2004; Shaw & Osborne 2011; Velásquez *et al.* 2018).

Melampsora lini, also known as flax rust, is an obligate parasite that is infecting populations of Lewis flax (*Linum lewisii*) throughout the subalpine region of the southern Rocky Mountains (Duggal *et al.* 2023; Jiranek *et al.* n.d.; Miller *et al.* 2022). *Linum lewisii* grows in the dry period between snowmelt and the monsoon season, making soil moisture highly influential on Lewis flax development (Iler, unpublished data). A rust infection can result in decreased flax seed yield and cause early leaf senescence as *M. lini* extracts nutrients from the flax plant (Duggal *et al.* 2023; Lawrence *et al.* 2007). The prevalence of rust infections varies widely between populations, with proximity to an infected plant having little impact on the likelihood of spread, and tends to have a greater impact on isolated populations of *L. lewisii* (Höckerstedt *et al.* 2022; Shaw & Osborne 2011). However, *M. lini* does not fare well over the winter, with only 20% of infections overwintering successfully (Lawrence *et al.* 2007).

Although it is clear that earlier snowmelt is impacting the phenology of plants (Iler *et al.* 2021; Inouye 2008; Rose-Person *et al.* 2024), little is known about the impact it has on the success of obligate pathogens present in those plant populations. Since overwintering of *M. lini* in the root systems of *L. lewisii* is fairly unsuccessful, the spread of *M. lini* is highly dependent on spores and direct transmission via wind (Lawrence *et al.* 2007; Miller *et al.* 2022). Weather patterns have a high potential to be impacted by environmental change (van den Berg & van den Bosch 2007; Miller *et al.* 2022; Shaw & Osborne 2011). The changing environment has potential to negatively impact both *L. lewisii* and *M. lini* with exacerbated drought conditions (Duggal *et al.* 2023; Iler *et al.* 2021; Miller *et al.* 2022; Shaw & Osborne 2011), so changes in infection rate could increase or decrease depending on who fairs worse under the new conditions. It is unknown if earlier snowmelt provides a less favorable environment for the

spread of pathogens, or if there is a higher risk of infection for *L. lewisii* due to a longer growth period. Understanding the prevalence in both treatments could provide insight into who's experiencing greater impacts.

In this study, we investigate the effect of early snowmelt on flax rust, *Melampsora lini*, within sub-alpine populations of Lewis flax, *Linum lewisii*. Additionally, we will investigate the consequences of rust infection on flax populations to understand how infection will vary between treatments and how infection affects the fitness of *Linum lewisii*. Using a field-based experiment, we ask the following questions: 1) How do demographic measurements like flowering success and number of flowers vary with infection status? 2) How does the prevalence and severity of rust vary between early snowmelt and control treatments? 3) How does reinfection vary between treatments?

METHODS:

Study Species:

Linum lewisii is a perennial plant in the Linaceae family. Their primary vegetative growth window occurs during the dry period between snowmelt and monsoon season. They grow from a branched caudex, sending up many leafy shoots that can vary in height (Addicott, 1977). *L. lewisii* peak flowering occurs in July, with tails bleeding into late June and early August (Figure 1). They quickly abscise their petals within 24 hours of flowering, but may produce many flowers throughout the season – making the flowering season substantial (Addicott, 1977). *Melampsora lini* is a fungal pathogen which is reliant on flax and linseed to carry out its life cycle (Lawrence et al., 2007). Infection is typically caused by wind dispersed spores, although it can occasionally overwinter in the plant's rootstock (Duggal et al., 2023; Ravensdale et al., 2011).

Snowmelt Plots:

I conducted this study in the summer of 2025 in the sub-alpine perennial research meadow on-site in the Rocky Mountain Biological Laboratory (RMBL) located in Gothic, Colorado. The plots were all within 250 m of each other within the research meadow. There were 8 plots total, grouped into blocks with one control and one experimental plot – resulting in 4 replicates for each treatment (Figure 1). I worked in 4 blocks established in 2021 by Dr. Amy Iler. Within each block were two plots, consisting of one early snowmelt treatment and one control plot. Each plot was trisected into 3 subplots, for ease of locating individual plants. These plots were set up in a naturally occurring meadow, meaning no additional planting was done.



Figure 1: Plot locations within research meadow. Pink are control plots, blue are early snowmelt plots. All spatial data gathered using a Trimble DA2 antenna.



Figure 2: Early snowmelt condition methods. The left shows shade cloths on snow in the early season, the right is post melt-out.

Early snowmelt was simulated by covering half the plots with shade cloths (Figure 2), increasing the heat absorption from the sun in those areas. In 2025, this caused them to melt out 11-17 (average = 14.5) days earlier than their control counterparts. As a result, peak flowering also occurs earlier; in 2024, peak flowering occurred 5 days earlier in the experimental plot than in the control plot (Figure 3; Iler and Simpson, unpublished data).

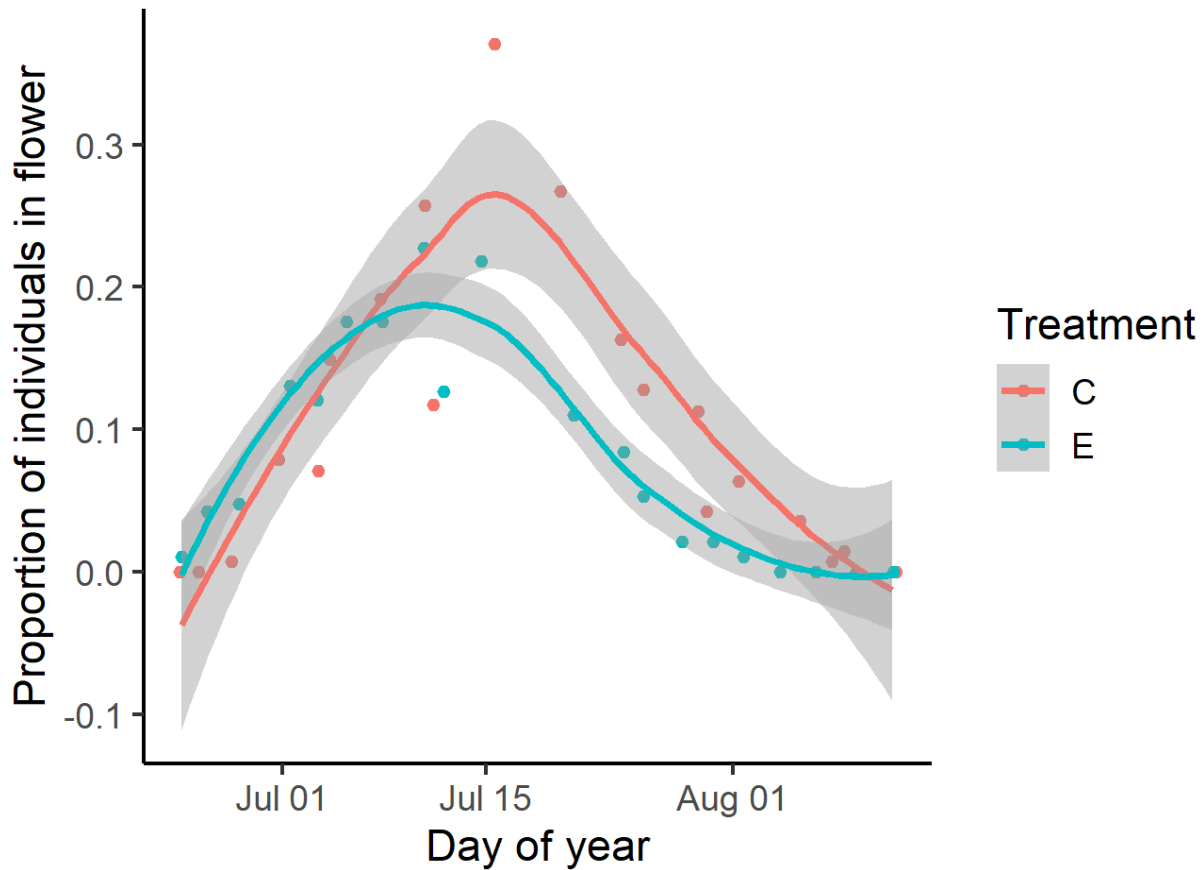


Figure 3: Scatter Plot graph showing flowering curve for control and experimental plots. The representative line is a LOESS curve.

Infection Rating System:

I conducted detailed rust surveys once to twice a week on a starting population of ~430 individuals. To identify and describe infected individuals within the plots, I ranked them using a class system described as follows:

- Class 0: Rust absent.
- Class 1: Rust present. Less than or equal to 5 pustules on plant
- Class 2: Rust present. More than 5 pustules, but less than 25% of plant infected.
- Class 3: Rust present. 25-50% of plant infected.
- Class 4: Rust present. 50-75% of plant infected.
- Class 5: Rust present. 75-100% of plant infected.

This protocol was adopted from Juliana Jiranek, who is a graduate student studying flax rust on populations of *Linum lewisii* at RMBL as well. Additionally, I recorded the location of the rust infection on the plant, specifically whether it was low or high on the stem. To prevent spread of infection, I used hand sanitizer in between each individual's assessment.

Demography:

I collaborated with the Iler lab to collect demographic data for *Linum lewisii*, which I later used to describe changes in growth and productivity with the presence of rust. I assisted in collecting demographic data, which occurred only once throughout the season – once vegetative growth plateaued and widespread flowering began. Within that, I collected size data of each individual, quantified by the number of stalks and the height of the tallest stalk. I recorded binomial flowering data for all tracked plants and, on flowering plants, the number of flowers (open, aborted, and buds) were recorded.

Data Analysis:

Rust presence was quantified using the ordinal scale above, and compared across treatment plots. Both treatment and rust presence were used as predictors for different analyses. Prevalence, reinfection rate, flowering probability and flower count were compared across treatments using generalized linear mixed effect models. Infection prevalence, reinfection rate, and flowering probability were modeled using logistic regressions. Flower count was modeled using a negative binomial regression. Reinfection was modeled using a non-parametric rank test (Wilcoxon). In each model, we included block as a random intercept term and plant height as a covariate (Table 1). We included height because we expected larger plants would be more likely to be infected due to greater surface area for catching wind-dispersed spores. To assess the rate of reinfection, we modeled the probability of plants infected in 2024 also being infected in 2025. Individuals from 2025 were included in this model if they had infections early in the season and low on their stalks. Statistical analysis was done using R and RStudio, utilizing the 'glmer()' function within the 'lme4' package.

RESULTS:

Using data from 427 individuals sampled, we tested response variables across two predictors to answer the proposed questions. Prevalence had a significant relationship with treatment, with probability of infection being lower in the early snowmelt treatment ($p < 0.05$). There was no observed correlation between treatment and severity of rust infection ($W = 3554$, $p > 0.05$). There was no significant relationship between reinfection and treatment ($z = 0.311$, $p > 0.05$). When comparing demographic measures, we found infected plants flowered more often than non-infected plants ($z = 2.584$, $p < 0.01$). The number of flowers produced was shown to have no significant relationship with infection status ($z = 0.336$, $p > 0.05$). We found infected plants were significantly larger than uninfected plants ($X_1 = 118$, $p < 0.05$). Size was a highly significant covariate in all models, with larger plants being more likely to be infected, more likely to flower, and more likely to produce more flowers.

Table 1: Summary of models run and their corresponding questions. Prevalence refers to the probability of infection at the plant level; severity refers to the ordinal-scale infection class; reinfection refers to the probability of an infected plant in 2024 being reinfected in 2025;

flowering refers to the probability of a plant flowering at all; and flower count refers to the total number of flowers produced.

Q#	Response	Predictor	Covariates	Model Type	P-value
1	Prevalence	Treatment	Block, height	Logistic Regression	0.0371
1	Severity	Treatment	NA	Wilcoxon	0.9911
2	Reinfection	Treatment	Block, height	Logistic Regression	0.7558
3	Size	Infection Status	Block, treatment	Linear Mixed Effect	< 2.2e-16
3	Flowering	Infection Status	Block, treatment, height	Logistic Regression	0.0329
3	Flower Count	Infection Status	Block, treatment, height	Negative Binomial	0.7553

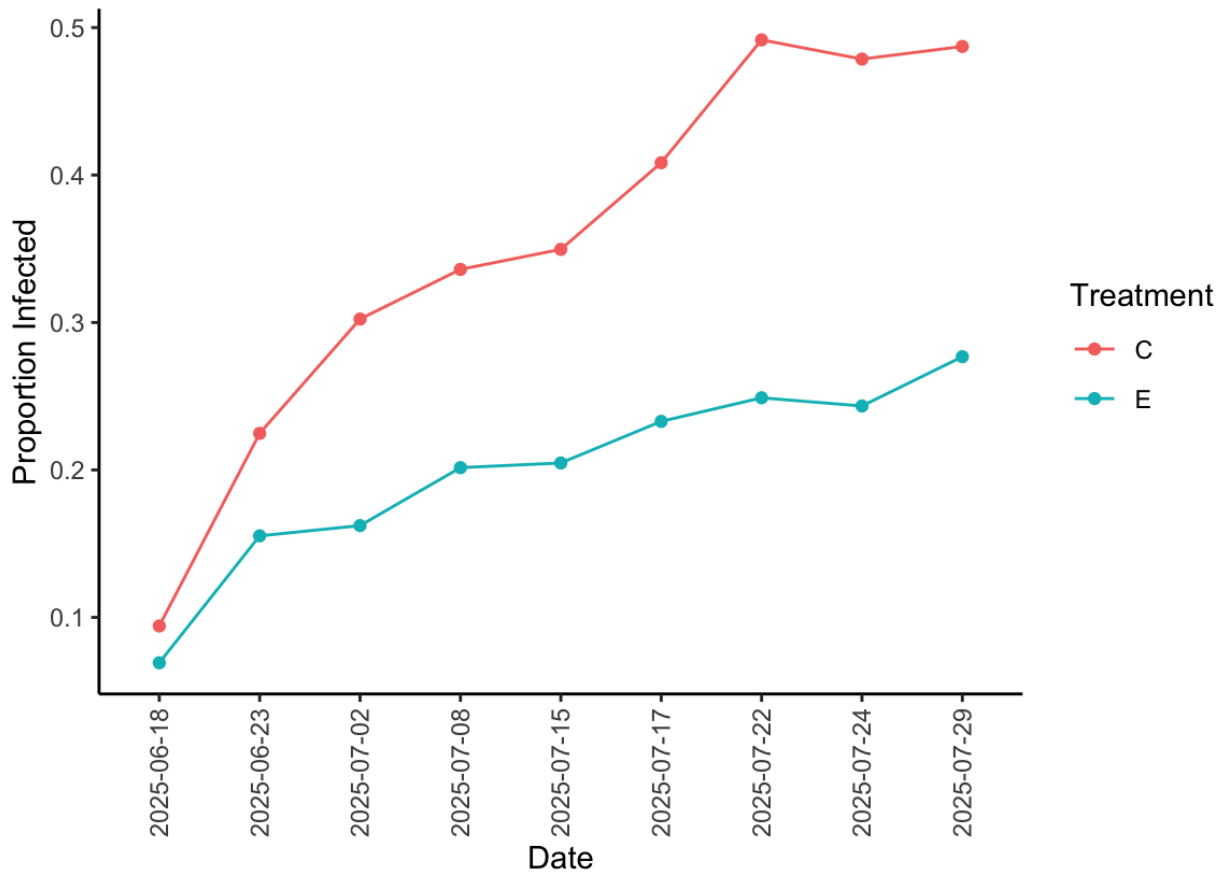


Figure 4: Scatterplot of prevalence over time. Proportions were calculated using the sum of infected individuals across all plots in a certain treatment and the total number of plants in said treatment, to get one proportion per survey round. Effects of treatment strengthened as the summer progressed, as seen by the steady deviation of the two lines.

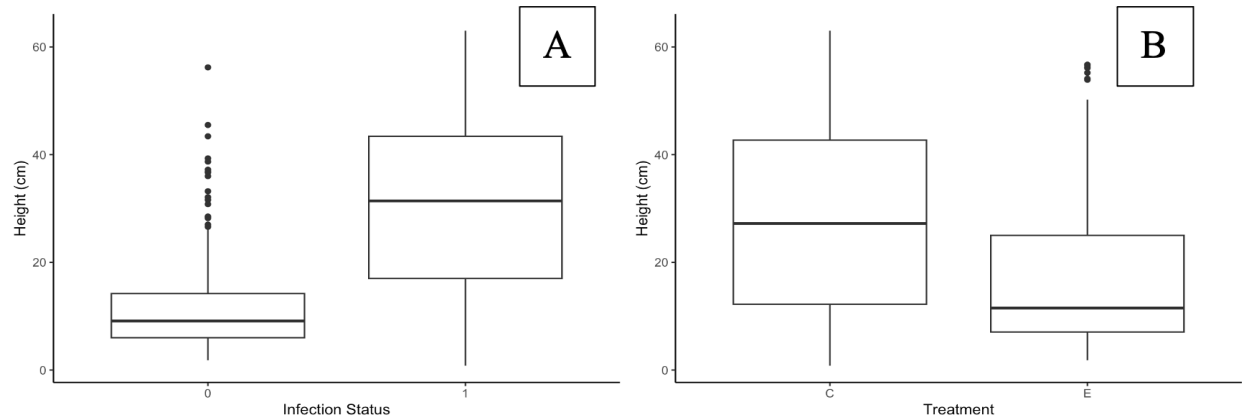


Figure 5: Higher infection prevalence in control plots can be explained in part by mean height being taller in control than in experimental plots. A) Mean height is greater for infected individuals than for non-infected individuals. B) Mean height is greater in the control than in the experimental plots. Individual plant height plotted for treatment and infection status.

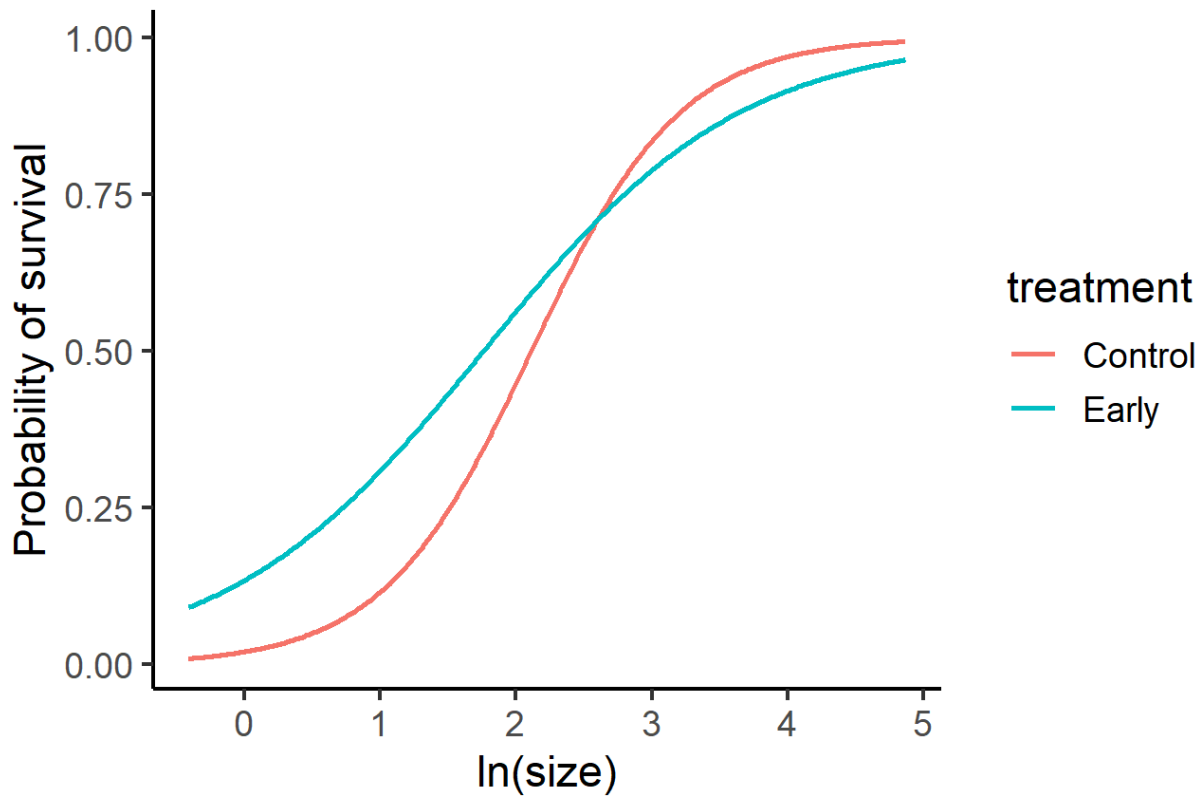


Figure 6: Survivorship curve for control vs $\ln(\text{size})$ and experimental treatments produced with previous years survival data. Shorter plants have higher survivorship in experimental treatments than in control, with the inverse being true for taller plants.

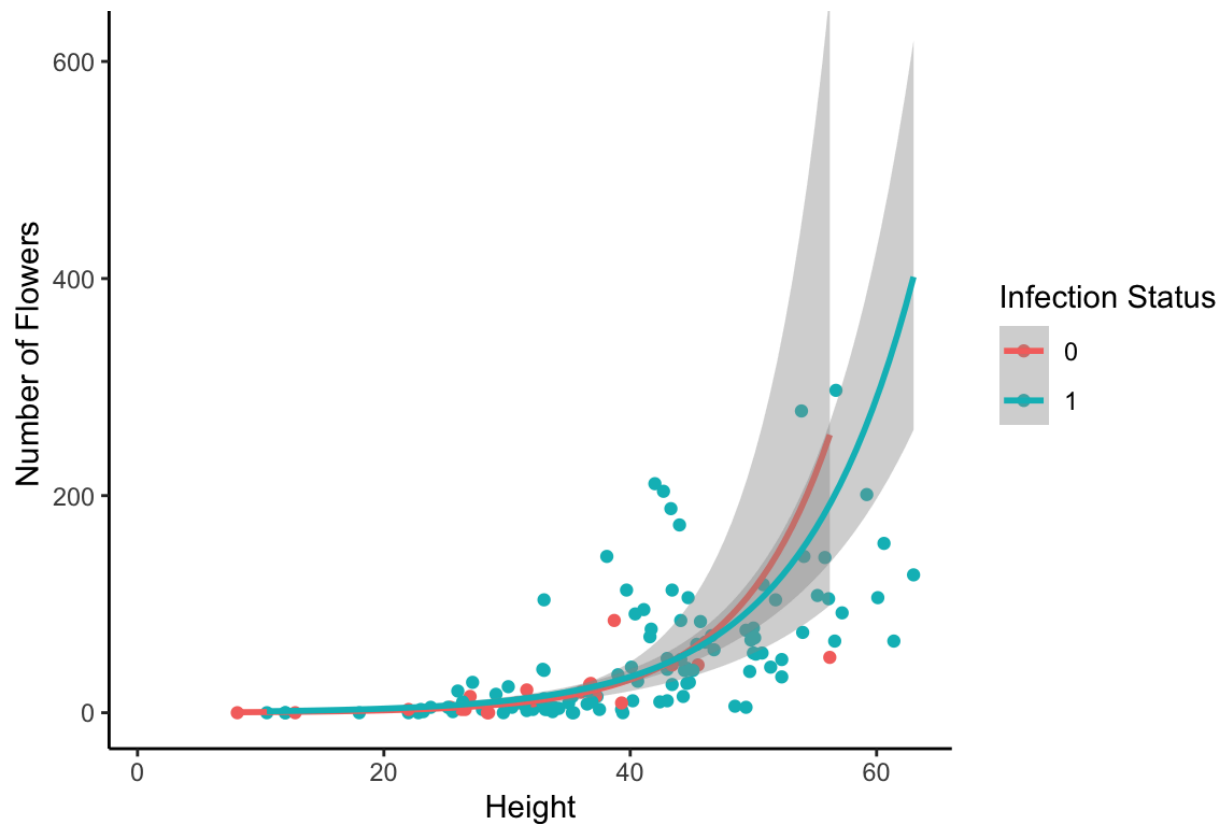


Figure 7: Curve of flower count for control and treatment conditions. No significant relationship between these factors ($p = 0.7553$).

DISCUSSION:

In this study we set out to understand how early snowmelt influences the prevalence, severity, and reinfection of *Melampsora lini* in subalpine populations of *Linum lewisii*. To understand how rust infections influence demographic metrics in *L. lewisii*, we compared infection status with likelihood of flowering and flower count – using height as a covariate for all models. Broadly, we found higher prevalence in control treatments and higher flowering rate in infection plants.

Across all findings, one aspect to keep in mind is the particularly dry weather conditions experienced in Gothic, CO during the 2025 field season (Rainfall deviation: *June 2025*: -0.18 in; *July 2025*: -1.24 in, from gothicwx.org). Drought stress on *Linum lewisii* was observed throughout the season, both with personal observations and mortality data, with the sample size dropping notably from 426 to 341 over the course of the data collection window (Iler & Simpson, unpublished). Drought conditions have the potential to induce stress on both pathogens and hosts (Velásquez et al. 2018). Since high humidity is known to benefit spore dispersed pathogens like *M. lini* (Lawrence et al. 2007; Romero et al. 2022), the dry conditions throughout the season could have had an equal impact on success of rust in all plots – effectively normalizing the effect of snowmelt across treatments.

Throughout the season, the prevalence of rust in the early snowmelt conditions was significantly lower than the control (Figure 4). Early in the season, differences in infection between treatments could be explained by plant size alone, with there being more large plants in the control plots and larger plants subsequently being more likely to be infected (Figure 5). However, as the season went on, the difference between treatments got stronger (Figure 4). In the end, there was evidence for effects of both plant size and treatment. These results support the initial hypothesis that early snowmelt would affect fitness of *M. lini* and thus affect infection prevalence. This could be due to the fact that the early snowmelt plots had a longer period of exposure to drought conditions, reducing microclimate humidity and the ability of the fungus to propagate on new plants.

Although prevalence significantly varied, treatment proved to have no significant effect on severity or reinfection. This implies that any climatic conditions which varied across treatments did not have a strong impact on what determines infection progression. Drought conditions could have equally impacted *M. lini* fitness across plots, reducing its success at proliferating beyond initial infection. Alternatively, there could be additional abiotic factors that remained consistent across plots which influence severity and reinfection more than the treatment.

Height was found to strongly influence the likelihood of rust infection in *Linum lewisii*, with larger plants being more likely to be infected than smaller plants. Since *M. lini* is known to be a primarily wind-dispersed fungal pathogen (Lawrence et al. 2007; Miller et al. 2022), the correlation between height and likelihood of infection could be explained by taller individuals having a greater area for spores to land on. Plants in the control plots were on average taller than those in the experimental plots (Mean Control = 15.39 cm; Mean Treatment = 8.95 cm), which could be attributed to variation in survivorship between treatments (Figure 6). Smaller individuals tended to be less likely to survive within the control treatments, and have a greater likelihood of being shielded from spores by surrounding taller plants (Figure 6, Iler & Simpson, unpublished).

The majority of the difference between the predictor and response variables across treatments could be attributed to variation in size across the sample populations. Even if climatic changes are not directly impacting plant physiology by changing growth and reproductive success, they may shift community demographics by altering the proportions of certain life stages within populations (Figure 6, Iler & Simpson, unpublished). This demographic shift could result in a new size structure for pathogens and other organisms to navigate within these ecosystems – resulting in a high potential for altered biodiversity (Iler et al. 2021; Mehring et al. 2020).

Infected individuals were more likely to flower than uninfected individuals, even after controlling for size and treatment. This could be due to the stress conditions brought on by an active infection (Lawrence et al. 2007). Stress factors like poor nutrients, high light intensity, and low temperatures are known to induce flowering (Wada & Takeno 2010). A rust infection could

induce the same physiological response as these conditions, as the pathogen extracts nutrients from its host.

Although the likelihood of flowering varied, infection seemed to have no influence on the number of flowers produced by a plant (Table 1, Figure 7). Since the determinants of the probability of flowering and the number of flowers produced are separate mechanisms within an individual, *M. lini* could have a strong influence on one but not the other. Flower production is heavily influenced by environmental and genetic factors, which could have a greater impact on flower count than infection status (Lemmon *et al.* 2016).

Demographic changes in plants due to climate change can have negative or positive effects on their relationships with pathogens. Regardless of the reason, mass physiological changes in plants on a community level will have varying effects on pathogen distribution and prevalence. In this case, it tended to work in favor of *L. lewisii* by shifting community demographics and reducing overall prevalence of infection. This will not always be the case, and additional research is required to understand how and why this relationship is shifting in this direction. There is no guarantee that this relationship will remain consistent, as new strains could evolve that are better suited for the altered environment (Velásquez *et al.* 2018).

Beyond the species level, if community demographics change with climatic conditions – there is potential for entire ecosystems to alter their dynamics. As new conditions emerge, plants, pathogens, and all members of an ecosystem will be required to adapt to fill new niches. Not only will plant-pathogen relationships vary, but keystone interactions could no longer function like they are supposed to. This shift will be a new frontier which requires immense monitoring to understand and describe the changes we will see.

RESOURCES:

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