**Predicting the effects of climate change on the cross-scale epidemiological dynamics of a fungal plant pathogen**

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**Abstract**

The potential for climate change to exacerbate the burden of human infectious diseases has been increasingly recognized, but less attention has been given to the effects of climate change on infectious diseases of plants. Plant pathogens are critical components of natural ecosystems, and also pose a serious threat to agricultural production and food security, reducing the yield of major crops by about 20% each year. We use the fungal pathogen ‘flax rust’ (*Melampsora lini)* and its subalpine wildflower host Lewis flax (*Linum lewisii),* to investigate the how climate change might affect the epidemiology and evolution of plant pathogens. By tracking the spread of disease within and between plants in populations spanning a large temperature gradient and incorporating these cross-scale transmission dynamics into an epidemiological model, we can predict how warmer temperatures might affect the speed and severity of flax rust epidemics. Combining these predictions with multiannual demographic data in an evolutionary model illuminates how climate change might alter flax rust virulence, and as a result drive further changes in epidemiological patterns. Insights from flax rust emphasize the importance of developing a mechanistic understanding of plant pathogen epidemiology and the value studying plant infectious disease in natural systems.

**Introduction**

To infer how climate change might affect the within and between host transmission dynamics of flax rust, we began by making observations of within-host disease spread in four *L. lewisii* study populations spanning a large elevation gradient. We then fit statistical models linking weather patterns to observed changes in infection intensity and simulated these models for future climate scenarios to infer effects on disease severity. Next, we sought to link within-host disease severity to transmission. We measured spore dispersal from individual infected plants and fit a tilted gaussian plume model (ref) that can be used to predict spatial patterns spore deposition from weather patterns and the infection intensity of the source plant. Using predictions of spore deposition generated from this model and observed patterns of infection within each of the four study populations, we fit a model linking spore deposition and environmental conditions to infection risk. Finally, we constructed a spatiotemporal epidemiological model of flax rust spread using the statistical models describing the effects of environmental conditions on within-host disease spread, spore dispersal, and infection risk. We validated this model against observed epidemiological patterns within each of the four study populations, and then simulated it for future climate scenarios to predict their associated effects on the population level disease spread of flax rust.

**Methods**

Overview

In this study, we aim to predict how climate change might alter the within and between host dynamics of flax rust spread. To observe how these processes play out in different climate conditions, we tracked four populations of *L. lewisii*  spanning an elevation gradient of roughly 1,000 meters. The large variation in weather between sites and across time allows for environmental drivers of transmission processes to be quantified via statistical modeling.

Within each flax population, we measured the location of all healthy and infected plants at the start of the observation period, tracked the spatiotemporal spread of infection, and simultaneously made detailed longitudinal observations of the within-host spread of *M. lini* in focal diseased plants. In addition to following the trajectories of infection intensity, we investigated two pathogen traits, replication and establishment, that may drive changes in infection intensity by making nested measurements within focal diseased plants. We used changes in pustule area as a proxy for replication, and changes in the number of pustules on a leaf as a proxy for establishment. We measured plant growth in both diseased focal plants and uninfected healthy focal plants to understand interactions between growth and disease progression. We identified environmental drivers of pathogen replication and establishment, plant growth, and change in infection intensity using generalized additive models.

To connect within-host infection intensity to spore dispersal, we deployed spore traps in arrays around focal diseased plants. We then counted the number of *M. lini* spores caught in each trap. We fit a tilted gaussian plume model describing spatial patterns of spore dispersal using this data, observed patterns of wind speed and direction, and measurements of source plant infection intensity and height. We then used this model to predict the number of spores deposited on each healthy plant in each study population (based on observed wind speed, locations of infected plants, and the infection intensity of those plants) between observations of the infection status of those plants. We then modeled the relationship between spore deposition, environmental factors, and the odds of a healthy plants becoming infected using a generalized additive model with a binomial link function.

In the final step of our analysis, we construct a spatiotemporal epidemiological model using the model of within host disease progression, the tilted gaussian plume model describing spore dispersal, and the model relating spore dispersal to the odds of infection. We initialized this model using the observed locations, heights, and infection statuses of plants in each study population. We then simulated the model using observed weather data and validated the results against observed epidemiological outcomes.

To infer how climate change might alter within-host patterns of flax rust spread, we simulated the model of within-host disease progression for a hypothetical plant starting with a light infection using observed weather data to generate predicted infection intensity trajectories. We validated these predictions against observed patterns of disease spread to ensure that model predictions qualitatively matched observations. Next, we repeated this simulation process while adding either 1.8 or 3.7 degrees Celsius to all temperature readings. To infer how climate change might alter the trajectories of flax-rust epidemics, we restimulated the spatiotemporal epidemiological model while again adding either 1.8 or 3.7 degrees Celsius to temperature readings.

Study system

*Linum lewisii*, commonly known as Lewis flax, is a short-lived perennial wildflower distributed across the western United States (ref). In our study area, *L. Lewisii* is most commonly found in open meadows, and occurs at elevations up to ~3,600 meters. M. lini is a fungal rust pathogen that infects species in the genus Linum. It completes all its life stages on its flax host, making it an ideal species for studying trade-offs in pathogen evolution. Urideniospores infect the stems and leaves of the host plant, forming bright yellow-orange pustules. The urideniospores propagated in these pustules are dispersed by the wind both within and between plants. This infection cycle repeats continuously over the course of a growing season (ref). The flax rust pathosystem is an excellent model for studying the effects of climate on the evolution and ecology of plant pathogens because transmission occurs across a wide range of environmental conditions, infection is easily observed in the field, and the immunological mechanisms underpinning resistance traits have been thoroughly characterized (ref).

Data collection

*a. study sites*

We selected four study sites in the Upper Gunnison Basin in Colorado, USA. As we intended for these sites to capture a large amount of variation in environmental conditions, the primary criteria we used for site selection was elevation. Mirroring patterns of mountain weather observed worldwide, (ref) elevation is correlated with a multitude of environmental factors in the Rocky Mountains. Predominant trends include a negative correlation between altitude and temperature, a positive correlation between altitude and precipitation (ref), and transitions in plant community composition (from ‘mountain shrub’ to ‘montane’ to ‘subalpine’ to ‘alpine’) with increasing altitude (ref).

Our sites span a large elevation gradient of approximately 1,000 meters that captures significant variation in environmental conditions. These sites should not be interpreted as a direct elevation for climate substitution, especially considering the significant changes in weather at each site that occur over the course of our observation periods, which generally ran mid-June to early August. The lowest site, ‘cement creek’ (hereafter “CC”), falls within a sage brush meadow on the boundary between the mountain shrub and montane vegetation zones at 2440 meters elevation. The second lowest site, ‘bus turnaround’ (“BT”), is situated in an open subalpine meadow at the base of Gothic Mountain at ~2940 meters. The second highest site, ‘gothic mountain’ (“GM”), is also in the subalpine zone on a steep hillside within a clearing in an evergreen forest on the lower slopes of Gothic Mountain at 3220 meters. The highest site, ‘high meadow’ (“HM”), is on an exposed meadow at the upper fringes of the subalpine zone on the upper shoulder of Gothic at 3410 meters.

*b. environmental condition monitoring*

At the beginning of each field season, we deployed environmental sensors to record longitudinal weather data at each of our sites. We recorded temperature, humidity, rainfall, leaf wetness (at 30 cm above ground), soil water content (at 10 cm below ground), solar radiation, and wind speed and direction (at ~1 meter above ground) every five minutes (see supplementary information for logger specifications). We used temperature and humidity data to calculate absolute humidity (see supplementary information).

*c. population mapping*

In order to uncover drivers of population level epidemiological dynamics, it is necessary to characterize the ‘landscape of susceptibility’ in the host population. For spatially structured infectious disease systems such as flax rust, this involves documenting not only the infection status of each individual and any likely covariates of contact rate or susceptibility (e.g. size), but also the spatial arrangement of individuals. To accomplish this, we established 10 by 20 meter transects at each of our field sites. We recorded the coordinates of each transect corner and the compass bearings associated with the transect axes (so that wind direction could be translated to the transect coordinate system) using a Garmin GPS unit (part #: 010-01735-10). At the beginning of each field season, we mapped the location of each plant within this transect, while also recording the height and infection status (healthy, diseased) of each plant. Plants with heights less than 5cm were denoted as seedlings. While other metrics of size such as stem count could be used to form a more complete picture of plant size, we chose to use height alone as a proxy for size as it was the only measure that we could feasibly record for the many hundreds of plants within each transect.

*d. epidemiological surveillance*

After documenting the initial epidemiological conditions, we tracked the spatiotemporal spread of flax rust within each transect. Approximately once per week, we inspected each flax individual in the transect for signs of disease, recording the location of any newly infected plants so they could be matched to a previously uninfected plant. Plants identified as infected in the initial population mapping and in these subsequent epidemiological surveys were marked with flags to ensure that they would not be repeatedly recorded as newly infected.

*e. diseased focal plants*

Within each transect, we designated a subset of diseased plants as ‘focal diseased plants’ and used them to make detailed measurements of within-host disease progression. All initially infected plants received this designation, and we continued to give this designation to newly diseased plants until we obtained approximately 25 infected focal diseased plants per site. We inserted metal tags into the ground at the base of these plants so they could be quickly and reliably identified. To facilitate longitudinal measurements of disease progression at sub-plant scales, we marked up to three infected stems on each focal diseased plant with pieces of colored flagging tape tied around the base of the stem. These stems were chosen haphazardly. On each of these stems (when possible), we marked the tip of one infected leaf with black ink. We preferentially marked leaves with one or a few pustules. Approximately once every three days, we recorded detailed observations of disease progression within each focal diseased plant:

To measure pustule growth, we photographed each marked leaf with a millimeter ruler in frame using a Cannon EOS Rebel T7i DSLR camera fitted with a Canon EF-S 35mm f/2.8 Macro IS STM lens and a Cannon MR-14EX II Macro Ring Lite. While achieving consistent photographic angles and lighting conditions is impossible in a field setting, we attempted to keep the leaf and the scale in the same plane while maintaining a perpendicular shooting angle. We also adjusted camera settings so that pustule boundaries could be clearly distinguished in images. We used ImageJ software to measure the maximum and minimum diameters of each photographed pustule, and calculated pustule area assuming pustules to be elliptical. To enable the size of individual pustules to be tracked across observations, each measured pustules in each image was labeled so that it could be re-identified in subsequent images based on its location on the leaf position relative to other pustules. We omitted data for any pustule that could not be confidently identified or whose borders became indistinct from other pustules. We marked a new leaf when we were unable to find the previously marked leaf, when the leaf was accidentally removed during data collection, or when the condition of the leaf deteriorated significantly. We suspect that either the presence of ink or our methods contributed significantly to the rate at which leaf condition deteriorated, but nevertheless we were able to observe the growth of many pustules over many weeks.

To measure the process of pustule establishment, we made use of these same photographs to measure changes in pustule number on each leaf by simply counting the number of pustules present. Distinguishing between pustules was often difficult because pustule growth often resulted in overlapping borders between pustules. In such cases, the number of pustules present in such a clump was approximated as the number of nearly-complete pustule outlines that likely once belonged to a single pustule. We only counted pustule number for photographs that covered the entire leaf surface.

To measure the intensity of infection at the whole-plant scale we multiplied (1) an estimate of the average number of pustules per infected leaf, (2) an estimate of the average length of infected tissue per infected stem, and (3) the number of infected stems to calculate a plant infection intensity metric. Because calculating (1) and (2) across all infected stems would not be feasible, we focused on the marked stems on each focal diseased plant. For each marked stem, we recorded the distance from the lowest (relative to the ground) pustule to the highest pustule and calculated (1) as the average of these measurements. Likewise, we counted the number of pustules present on a haphazardly selected leaf in the middle of the region of infected tissue on each marked stem and calculated (2) as the average of these counts. We did not use the same leaf for each observation as the middle of the region of infected tissue changed due to disease spread. To obtain (3) we counted the number of infected stems. We also measured the height of each diseased plant at each observation to analyze the effects of environmental conditions and infection intensity on plant growth.

*f. healthy focal plants*

As we wished to investigate patterns of growth in both healthy and infected plants, we designated certain uninfected plants as healthy focal plants, and measured their height whenever we measured the height of focal diseased plants. To control for potential effects of stem marking on the growth of diseased focal plants, we made these same markings on healthy focal plants. Healthy focal plants were selected haphazardly, and we added new healthy focal plants over time to keep the number of diseased and healthy focal plants approximately equal. At some sites, the number of healthy focal plants fell towards the end of the observation period as many healthy focal plants became infected. When this occurred, we stopped recording data for the plant, and in some cases designated it as a diseased focal plant.

*f. spore deposition*

To measure the distribution of spore deposition from an infected plant, and how it relates to the infection intensity of the source plant and wind patterns, we deployed spore traps in arrays around a subset of focal diseased plants. We chose focal disease plants that were as removed as possible from other diseased plants to minimize the number of spores originating from other plants that would be caught in the spore traps. These spore traps consisted of a ~2 cm2 section of Scotch Permanent Clear Mounting Tape (part #: ‎MT76272-5) affixed to plastic backing with double sided tape. These spore traps were secured into the ground with ~5 cm nails. We deployed these traps at distances of either {~5cm} (traps at ~5cm were placed as close to the plant as possible), {~5 and 25 cm}, {~5, 25, and 50 cm}, or {~5, 25, 50, and 100 cm} in each of the four directions corresponding to the axes of the transect in which they were located. The traps were left in the field for approximately one week, and then collected. The sections of mounting tape were transferred to microscope slides and sealed in place using clear packing tape. We then used a light microscope to count the number of spores present in a known area. We counted spores in an area of 0.8 cm2 for most spore traps that contained few or no spores, but we counted a minimum area of 0.1 cm2 for all spore traps, including those with greater than 10,000 spores per cm2. *M. lini* spores were identified visually using a slide prepared with spores as a guide. The spores matched descriptions in the literature (refs). We did not observe any fungi other than *M. lini* in our around our transects, so we can be reasonably certain that our counts represent only *M. lini* spores from infected focal diseased plants.

Statistical Analyses

*a. Plant growth and within-host disease spread*

To infer how different weather factors affect plant growth, pustule growth, pustule establishment, and changes in infection intensity, we fit generalized additive models. We used longitudinal observations of plant height in healthy and diseased focal plants to fit the model of plant growth, and longitudinal observations of within-host disease spread at various scales in focal diseased plants to fit all other models. Because the observation periods associated with our measurements of these processes varied, we formatted the response variables in change-per-day units: For our analyses of plant growth, pustule growth, pustule establishment, and infection intensity progression we used change in plant height per day, change in pustule area per day, change in the number of pustules present on a leaf per day, and change in infection intensity per day respectively as the response variables.

To infer the effects of weather variables, we included smooth terms for these factors as predictors in each model. For each observation of change in plant height, pustule area, pustule number, or infection intensity, we determined the start and end timepoints of the observation period to extract the corresponding weather data. For observations of plant height, we used 12:00 P.M. on the day of the observation as the start/end timepoints of the observation period. For observations of pustule area and pustule number, we used photograph timestamps as the start and end timepoints. For observations of infection intensity, we used the average of the timestamps of photographs taken of pustules on that plant’s leaves as the start and/or end timepoints if such photographs were taken, because all data for an individual diseased focal plant was generally collected at once. If such photographs were not taken, we used the mean timestamp of all photographs taken at that plant’s site on the observation date as the start and/or end timepoint. To focus our analysis on fine-grained relationships between weather and within-host disease spread, we discarded data corresponding to observation windows of eight or more days. Using the start and end timepoints of the observation period as bounds, we extracted the following covariates from the weather data: temperature (mean, maximum, and minimum), absolute humidity (mean, maximum, and minimum), mean solar radiation, mean rainfall, and mean leaf wetness.

We included smooth terms for all these weather variables as predictors in each model, along with smooth terms study site and plant identity (i.e. the plant on which the pustule or pustules were observed in the case of the analyses of pustule growth and establishment) as random effects (accomplished by setting bs=”re” in the s() function in mgcv). In each model we also included a term that accounted for the value of the previous observation to capture any ‘allometric’ effects. In the model of change in plant height per day, this term was a full tensor smooth product of last observed height and infection intensity. In the model of change in pustule area per day, this term was a smooth of last observed pustule area. In the model of change in the number of pustules on a leaf per day, this term was a smooth of the last observed number of pustules on that leaf. In the model of change in infection intensity per day, this term was a full tensor smooth product of last observed infection intensity and last observed maximum plant height.

We fit these models using the gam() function in the mgcv R package and the restricted maximum likelihood parameter estimation method. We used gaussian response distributions with identity link functions. To implement variable selection so that insignificant predictor terms would be effectively removed from the modes, we used the double penalty approach of Mara and Wood (via the ‘select=TRUE’ option in the mgcv gam() function).

The coefficients of the fit models can be used to infer the effects of weather factors on plant growth and within host disease spread. To translate these inferences into predictions about how climate change might affect plant growth and within-host disease spread, we simulated trajectories of plant growth, pustule area, number of pustules on a leaf, and infection intensity under various climate conditions. For each class of simulation, we started by defined a hypothetical starting state. For simulations of plant growth, this was a 15cm tall uninfected plant (the plant was assumed to remain uninfected for the entire simulation). For simulations of pustule growth, this was a pustule with area .1cm2. For simulations of number of pustules per leaf, this was a leaf with one pustule. For simulations of infection intensity, this was a 15 cm tall plant with infection intensity 0.1. Next, we simulated 100 trajectories using observed weather data for each site. After confirming that these simulation results qualitatively recapitulated observed patterns, we simulated trajectories for two climate change scenarios. In the mitigated climate change scenario, we added 1.8 degrees Celsius added to temperature readings of observed weather data at each site. Likewise, for the unmitigated climate change scenario, we added 3.7 degrees Celsius to observed weather data. These temperature additions correspond to the predicted change in temperatures corresponding to mitigated and unmitigated climate change scenarios (source). Simulations were performed via bootstrap simulations of fit models. We simulated trajectories in time steps of one day.

*e. Spore dispersal*

To infer how spore deposition is related to source plant infection intensity, wind speed, and wind direction, we fit a tilted gaussian plume model (source) to our spore deposition data. The equation specifying the tilted gaussian plume model is as follows:

This equation describes the concentration of spores deposited at a given point () as a function of wind speed (), the source concentration of spores (I), and the coordinates of the point (X,Y), relative to the source. The coordinate system has the source at the origin, the X-axis parallel to the wind direction (with wind flowing in a positive direction), the Y-axis perpendicular to the X-axis on the plane defined by the ground (assumed to be flat), and the vertical Z-axis orthogonal to the X and Y axes. The shape of the three dimensional spore plume emanating from the source is defined by , along with constants specifying the falling velocity of spores , the height of the source (), and the standard deviation of spore dispersion is the horizontal (Y) and vertical (Z) directions­­.

*f. Infection*

*Epidemiological model*

*a. overview*

*b. structure*

initial conditions

spore dispersal

infection

infection intensity increase

growth

*c. simulation*

**Results**

Growth

Within-host processes

*a. growth*

*b. pustule size*

*c. pustule number*

*d. infection intensity*

Transmission

Epidemiological dynamics

**Supplementary Information**

Environmental condition monitoring equipment

We recorded temperature and humidity using HOBO Temperature/RH data loggers (part #: MX2301A) enclosed in solar radiation shields. These loggers were mounted approximately 30cm above ground level on wooden stakes. We recorded rainfall, leaf wetness, soil water content, solar radiation, wind speed, and wind direction using sensors connected to HOBO Micro Stations (part # H21-USB). Rainfall was measured using Davis (0.2 mm) Rain Gauge Smart Sensors (part # S-RGF-M0002). Leaf wetness was measured using ONSET Leaf Wetness Smart Sensors (part #: S-LWA-M003). Soil water content was measured using ONSET 10HS Soil Moisture Smart Sensors (part #: S-SMD-M005) that measure water content in an approximately 1 liter volume of soil. Solar radiation was measured using ONSET silicon pyranometers (Part# S-LIB-M003). We leveled these sensors to ensure accurate readings. Wind speed and direction were measured using Davis Wind Speed and Direction Smart Sensors (part #: S-WCF-M003).

Absolute humidity

To calculate absolute humidity (in terms of g H2O per cubic meter of air) from our observations of temperature and humidity, we began with the ideal gas law where P is pressure, V is volume, n is the number of gas molecules in mols, R is the universal gas constant, and T is the temperature in Celsius.

By setting , substituting in the value of R, and rearranging variables, we can solve for :

To solve for as the number of mols of water present in the gas, we need to substitute the partial vapor pressure of water, , for P. We calculate the partial pressure of water by multiplying that saturation vapor pressure of water, , given by Teten’s formula (ref) by the relative humidity, , expressed as a percentage:

By substituting s3 and s4 into s2, we obtain the number of mols of water in 1 cubic meter of air as:

By multiplying the right side of this expression by the molecular weight of water, 18.02g/mol, and simplifying, we finish our derivation of absolute humidity as a function of temperature and relative humidity:

We do not intend for these sites to be interpreted strictly as representing a gradient of “hot and dry” to “cool and wet” conditions. Rather, because our observations do not completely support this ‘altitude for climate’ simplification, and because environmental conditions varied significantly across the period of flax growth and flax rust spread at all four sites,