High temperatures reduce growth, infection, and transmission of a naturally occurring fungal plant pathogen

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Abstract

Climate change is rapidly altering the distribution of suitable habitats for many species as well as their pathogenic microbes. For many pathogens, including vector-borne diseases of humans and agricultural pathogens, climate change is expected to increase transmission and lead to pathogen range expansions. However, if pathogens have a lower heat tolerance than their host, increased warming could generate 'thermal refugia' for hosts. Predicting the outcomes of warming on disease transmission requires detailed knowledge of the thermal tolerances of both the host and the pathogen. Such thermal tolerance studies are generally lacking for fungal pathogens of wild plant populations, despite the fact that plants form the base of all terrestrial communities. Here, we quantified three aspects of the thermal tolerance (growth, infection, and propagule production) of the naturally occurring fungal pathogen *Microbotryum lychnidis-dioicae*, which causes a sterilizing anther-smut disease on the herbaceous plant *Silene latifolia.* We also quantified two aspects of host thermal tolerance: seedling survival and flowering rate. We found that temperatures >30 °C reduced the ability of anther-smut spores to germinate, grow, and conjugate *in vitro*. In addition, we found that high temperatures (30 °C) during, or shortly after the time of inoculation strongly reduced the likelihood of infection in seedlings. Finally, we found that high summer temperatures in the field temporarily cured infected plants, likely reducing transmission. Notably, high temperatures did not reduce survival or flowering of the host plants. Taken together, our results show that the fungus is considerably more sensitive to high temperatures than its host plant. A warming climate could therefore result in reduced disease spread or even local pathogen extirpation, leading to thermal refugia for the host.

Introduction

Earth's climate is warming faster than many climate models initially predicted (IPCC 2022), and these changes in temperature are likely to shift species interactions (Gilman et al. 2010), including host-parasite interactions (Budria and Candolin 2014). This is because temperature plays a critical role in the development, expression, and timing of both host and pathogen life history traits (Verant et al. 2012, Mordecai et al. 2013, Shocket et al. 2018). Indeed there is now a growing body of work showing that warming temperatures are likely to increase transmission and the geographic distribution of many important vector-borne human pathogens (Mordecai et al. 2017, Shocket et al. 2020) and pathogens of agricultural crops (Rossi et al. 2001, Bebber et al. 2013). However, not all pathogens will necessarily benefit from a warmer climate. For example, the invasive fungal pathogen of amphibians, *Batrachochytrium dendrobatidis (Bd),* is particularly sensitive to high temperatures (Nowakowski et al. 2016). A useful, recent framework for predicting organismal responses to climate change is the thermal mismatch hypothesis (Cohen et al. 2017). If pathogens have a lower thermal maximum (the maximum temperature for survival and growth and reproduction) than their hosts, then warming temperatures can increase the disease-free niche space for the host, generating 'thermal refugia' for hosts (Gsell et al. 2023). However, predicting thermal refugia requires knowledge of the thermal optima and maximum of both host and pathogen (Mordecai et al. 2013).

Plants form the base of all terrestrial food chains; understanding and predicting how climate change will affect plant-pathogen interactions is critically important. Yet, to date, most measures of plant-pathogen thermal tolerance have focused on agricultural pathogens (Rossi et al. 2001, Raftoyannis and Dick 2002, Chaloner et al. 2021, Shah and Bergstrom 2000), where there is growing evidence that warming will exacerbate disease spread (Bebber et al. 2013). In

contrast, much less is known about the thermal tolerance of wild plant pathogens (Robin et al. 2017), despite the fact that pathogens can have strong impacts on plant population growth (Alexander 2010, Gilbert 2002), alter competitive interactions (Mordecai 2011), and affect community composition (Bever et al. 2015). In many cases, only *in vitro* pathogen growth rates are measured (Robin et al. 2017), which often have a wider thermal tolerance range than *in planta* traits (Chaloner et al. 2021), or knowledge of pathogen thermal tolerance is inferred from field distribution data (Penczykowski et al. 2015, Scala et al. 2019, Dudney et al. 2021).

Microbotryum lychnidis-dioicae is a naturally occurring fungal pathogen of the herbaceous plant *Silene latifolia* and has become a model system for disease ecology (Bernasconi et al. 2009). Infected plants produce spores instead of pollen, resulting in host sterility. Several lines of evidence suggest that *M. lychnidis-dioicae* has a lower heat tolerance than its host. First, while the host plant *Silene latifolia* occurs across a broad range of latitudes and elevations, disease prevalence is higher in cooler climates and at higher elevations (Hood et al. 2010, Abbate and Antonovics 2014, Bruns et al. 2018, Kido and Hood 2020). Second, in greenhouse settings, high heat has been observed to cause temporary host recovery, enabling the host to produce fertile flowers (Elmqvist et al. 1993). However, a controlled study of the effect of temperature on infection, growth and transmission ability of this model plant-pathogen system has never been carried out. Furthermore, it is unclear whether the 'heat-curing effect' is limited to a greenhouse environment or also occurs in natural field settings.

Here we used a combination of lab, greenhouse, and field experiments to measure the relationship between temperature and the following three key life history stages of *M. lychnidis-dioicae: in vitro* performance of the spores*,* seedling infection ability, and the ability to sporulate in the host's anthers. We also quantified host survival and reproductive ability across these same

temperatures. We hypothesized that high temperatures would reduce the overall ability of the pathogen to germinate, infect and reproduce within its host, without reducing host survival and reproduction. Our results show that temperatures > 30 °C strongly reduced all three measures of pathogen performance. However, host survival and flowering ability did not decline at high temperatures, suggesting that climate change could substantially alter host-pathogen interactions in ways favorable to the host.

Materials and Methods

Study System

Silene latifolia (formerly *Silene alba*; Caryophyllaceae) is an herbaceous short-lived perennial plant native to Europe. It was introduced to North America in the mid 1800s and is now found across the east coast (Taylor and Keller 2007), where it tends to grow in patchy roadside metapopulations (Alexander and Antonovics 1988). The plant is dioecious; individual plants are either pollen-producing males or seed-producing females.

Microbotryum lychnidis-dioicae (Denchev et al. 2008) is a fungus in the Basidiomycota that causes a systemic anther-smut disease on *Silene latifolia*. It is a naturally occurring endemic pathogen in both the native range in Europe and in North America where it was introduced (Fontaine et al. 2013). The fungus hijacks the plant's flowers for its own reproduction, producing diploid teliospores in the anthers, and preventing pollen production. Infected female plants also produce spore-filled anthers, and the ovary is sterilized (Schäfer et al. 2010). Infection does not cause plant mortality, but the fungus is systemic and can spread throughout the plant, potentially affecting all flowers, rendering plants completely sterile. Transmission of the spores to new hosts

can occur through pollinator movement and highly localized wind transmission (Bruns et al. 2017, Roche et al. 1995). Before teliospores of *M. lychnidis-dioicae* can infect *S. latifolia*, they germinate and go through meiosis producing haploid sporidia that can reproduce asexually by budding. Each sporidium is one mating type, either a1 or a2. Prior to infection, two sporidia of opposite mating types must conjugate to produce an infectious hypha (Giraud et al. 2008).

Overview of lab and field experiments

We carried out three different experiments to determine the effect of temperature on the expression of *M. lychnidis-dioicae* and *S. latifolia* life-history traits. In Experiment 1, we assayed metrics of teliospore germination, haploid sporidial growth, and conjugation rates *in vitro* under a range of temperatures from 14-30 °C. In Experiment 2, we measured pathogen infection rate of *S. latifolia* seedlings, and survival of uninoculated seedlings under three temperatures (24, 26, and 30 °C) with a greenhouse experiment. In Experiment 3, we measured the expression of disease in infected adult plants in a field setting over a six-month period and analyzed the relationship between average high temperature and disease expression.

Experiment 1: Effect of temperature on spore traits *in vitro*

Isolating strains and mating types: Three strains of *M. lychnidis-dioicae* were isolated from a single infected population of *Silene latifolia* in June 2020 in Giles Co, VA (37.32867 N - 80.48812 W). Each strain used in this study was isolated from a single infected bud, each from a separate plant. Dilution plating was used to generate two haploid sporidial lines from each strain: one a1 mating type line and one a2 mating type line (see Appendix_S1 for details). In total, this

process produced six sporidial cultures: both the a1 and a2 mating types for three original infected plants.

Pathogen response to temperature *in vitro*

We measured three pathogen traits *in vitro:* teliospore germination, sporidial growth, and conjugation. All traits were assayed at 14 different temperatures (every 2 °C from 4-30 °C) in three Percival incubators (model I30BLLC8) with a 16-hour day and 8-hour night. Nighttime temperatures were set at 2 °C lower than the day temperature. Three temperatures were assayed in each experimental run, and experimental runs were repeated with different combinations of assay temperatures (order randomly determined) until every temperature had been assayed in two experimental runs. At each temperature within each experimental run there were three technical replicates (i.e., different plates) of each strain.

Teliospore Germination: We collected fresh teliospores from infected flowers within a common garden experiment in Beltsville, MD (see Experiment 3 below for details). Infected plants in the garden were originally inoculated with a mix of all three *M. lychnidis-dioicae* stains. Teliospores were diluted in sterile DI water to a concentration of 500 spores/μL, and 200 μL of this solution was then transferred onto a 1.5% water agar plates. Plates were incubated for 20 hours before imaging at 10X magnification on a light microscope (Leica DMi1). The number of germinated teliospores were counted manually in five fields of view on a Leica MC170 camera.

Colony growth: Sporidial viability and growth was assayed as the number of individual sporidial colonies visible after 7 days on 1% PDA (Potato Dextrose Agar; Difco^{TM}). Each sporidial line was diluted in sterile DI water to a concentration of 1 sporidia/ μLand 100 μLs

were plated onto 1% PDA (approximately 100 sporidia per plate). Plates were incubated for one week and then the number of visible colonies were counted manually.

Conjugation rate: For each strain, a solution was made containing a 50:50 mix of both a1 and a2 haploid sporidia at concentration of 100,000 spores/μL suspended in double deionized sterile water. Six droplets of the conjugation mix were pipetted onto a detached *S. latifolia* leaf suspended on a 1.5% water agar plate and incubated for 20 hours. After the incubation droplets from a single leaf were combined onto a glass slide with 1 μL of Lactophenol-Cotton Blue stain and a coverslip. Slides were imaged at 40X magnification on a light microscope (Lecia DM1000 LED) with a Leica MC170 HD camera. The number of single and conjugating sporidia was counted manually across 4 images per slide.

Statistical methods, Experiment 1

All statistical tests were run in R version 4.1.2. We used generalized additive models (GAM, package 'mgcv'; Wood 2011) to model the relationship between temperature and the three *in vitro* traits measured in Experiment 1, because this approach allowed more precise estimates of the non-linear relationship. Temperature replicate was included as a random factor in all models. To avoid overfitting, we varied the number of knots and visually inspected the change in fit. We then chose the minimum knot number that showed a smoothed, single peaked curve that did not substantially change with additional knots (See Appendix S1: Figure S1).

For growth, we included mating type and strain as additional linear predictors in the model. For conjugation rate, strain was included (mating type was not because both mating types were required for each conjugation event). Strain was not included in the germination model

because the teliospores were of unknown strain. We tested each effect in the model with likelihood ratio tests.

We used the 'predict' function to generate predicted trait values and 95% prediction confidence intervals for the measured temperature interval between 4-30 °C. The lower and upper temperature thresholds for each trait were defined as the temperature where the lower 95% confidence interval first went below zero on both sides of the temperature curve. The optimum temperature was defined as the temperature at which the focal trait had the highest predicted value.

Experiment 2: The effect of temperature on infection rate of seedlings

To test whether high temperatures resulted in reductions in infection *in planta* we set up a seedling inoculation experiment. We used incubators to manipulate the temperature at inoculation or four days after inoculation. In total we had five treatment groups: control-24 °C, 26 °C immediately at inoculation, 26 °C four days delayed, 30 °C immediately at inoculation, 30 °C four days delayed. For the immediate treatments the seedlings were heated to their treatment temperature for four days starting at inoculation and then moved to a 24 °C incubator. For the delayed treatments the seedlings were kept at 24 °C starting at inoculation for four days then moved to the treatment temperatures for four days. Within each of the five temperature treatments we had an inoculated and mock-inoculated group. The mock-inoculated group was used to assess the impact of temperature on the host plant in the absence of disease.

Seeds were planted in 148mm culture dishes (Falcon) containing 1% water agar. We used seeds from four different greenhouse reared full-sib families of *S. latifolia,* originally collected from Giles, Co. VA. These families were chosen because they previously had been shown to be

highly susceptible to anther-smut at the seedlings stage (Slowinski et al., unpublished data). Each culture dish contained 30 seeds of each family (120 seeds per dish) and we planted 3 dishes per temperature-treatment*inoculation-treatment (4 families * 30 plants per dish * 3 replicate dishes * 5 temperature treatments * 2 inoculation treatments = 3,600 seeds). Culture dishes were placed at a 12-hour day/night 24 °C/ 22 °C cycle in Percival incubators (model I30BLLC8) for one week.

Inoculations were carried out 7 days after planting. Incubator temperatures were adjusted to treatment temperatures the day of inoculation. We diluted fresh sporidia in distilled water from each of the three *M. lychnidis-dioicae* strains (a1 and a2) used in Experiment 1 to a standard concentration of 5,000 sporidia/μL. These were combined in equal proportions to make a single inoculation mixture of both a1 and a2 mating types of all three strains. We applied 3 μL of inoculation solution to the apical meristem of each seedling in the inoculation treatment. Seedlings in the mock-inoculation group were inoculated with 3μL of sterile double-deionized water.

On day 15, plates were removed from the incubators and 15 plants per family per plate (total 1,800 seeds) were transplanted into 1.5 x 8.25" Cone-tainers (Stuewe and Sons Inc) filled with Sunshine Mix #1 (Sungrow Horticulture). Cones were placed in racks in a blocked randomized design. After transplanting, the seedlings were kept in a greenhouse mist room for 2 weeks to allow the young plants to root, then moved into a greenhouse with 16-hour days. Plants were reared to flowering and then scored for presence of spores in their anthers. We also recorded any mortality, classified as plants with no green leaves left.

Statistical Methods, Experiment 2

To determine whether temperature affected infection rate, we used a generalized linear model with a binomial error structure. We included temperature treatment, plant family and replicate culture dish as linear predictors. Only plants in the inoculated treatment were included. Since initial model checking indicated overdispersion we used a quasibinomial model with a dispersion parameter of 1.8345. We first tested for an interaction effect between temperature*family, using a likelihood ratio test to compare the fit of models with and without the interaction effect. The interaction was not significant and was dropped from the final model, which included only main effects (see results). We visually checked the final model to confirm assumptions of homoscedasticity and normality. We tested the significance of each predictor in the final model using likelihood ratio tests.

To determine which of the temperature treatments differed significantly from the control treatment of constant 24 °C, we ran a one-way ANOVA on the proportions and followed up with a post-hoc Dunnett's test.

To determine whether temperature treatment affected seedling survival, we used a binomial glm on the whole data set, with temperature, inoculation treatment, plant family, and replicate culture dish as linear predictors. We started with a full model that included main effects and all pairwise interactions and removed any non-significant interaction terms (see results). We tested the significance of each predictor in the model using likelihood ratio tests.

Experiment 3: Effect of temperature on disease expression of infected plants in the field

We inoculated *Silene latifolia* plants with a mixture of the same three *Microbotryum* strains used in experiments 1 and 2 and reared them in the greenhouse. When the plants flowered, we discarded the healthy plants and selected 178 infected plants and transplanted them

into a common garden at the Maryland Agricultural Experiment Station (MAES) in Beltsville, Maryland. Infected plants were transplanted into 1-gallon pots filled with Sunshine mix 160 (Sungrow Horticulture). Pots were placed in 15 rows (spaced 3m apart), with 12 pots per row (spaced 3.75m apart). Each pot was sunk 6 inches into the soil and hooked up to an irrigation dripline. Pots were placed into the field on May 17, 2021. Every two weeks from May 17, 2021 until November 18, 2021 each plant was scored as flowering or not, as well as for the presence of spore-producing flowers. A local weather station at MAES collected daily information on the local maximum and minimum temperatures.

Statistical Methods: Experiment 3

Out of the 178 infected plants placed into the field, 6 never produced infected flowers and were excluded from the analysis. We defined the proportion infected as the proportion of plants producing at least 1 infected flower out of the total number flowering during a particular time point. We calculated the average high and low temperatures for the 7, 10, or 14-day period prior to each data collection time point using the MAES weather station data. To determine which temperature time-window (7 to 14-day average) was the best predictor of disease expression, we fit individual GAMs (Wood 2011) with a single temperature time-length as a smoothed predictor and compared proportion of deviance explained and the Un-biased Risk Estimator (UBRE; Wood 2006). We found that a 14-day average high temperature explained the most deviance and had one of the lowest UBRE scores (Appendix S1: Table S2).

Once we identified the best-fit temperature time window, we used this to determine the 'cutoff' high temperature that resulted in a decrease in infected flowers (i.e., an increase in 'curing'). To do this, we broke the data set into sets at a cutoff temperate and fit two lines (using

ordinary linear regression) for the data set before and after the cutoff. We recorded the total residual standard error across both models. Then we repeated this process with a range of cut-off temperatures and found the cutoff temperature that minimized the total residual standard error.

To determine how high heat impacted host plant flowering rate, we used linear regression to assess the relationship between the average 14-day high temperature and proportion of plants flowering.

Results

Experiment 1: Effect of temperature on spore traits *in vitro*

Temperature significantly affected germination rate, growth of colonies after one week, and conjugation rate (Table 1, Figure 1). All traits declined at high temperatures (>28 °C). However, traits varied in their sensitivity to heat, with the upper temperature threshold for germination rate > growth rate > conjugation rate (Table 1). Pathogen strain had a significant effect on colony growth (df=2, F=6.418, p=0.0018, Appendix S1: Table S1) and a marginally significant effect on conjugation rate (Appendix S1: Table S1). Estimates of thermal maxima were robust to variation in the number of knots used in the gam fits (Appendix S1: Figure S1).

Experiment 2: The effect of temperature on infection rate of seedlings

A total of 706 plants flowered and were scored for infection. Of these, 278 (39%) were infected. In the mock inoculation group, a total of 406 plants flowered and 2 plants became infected (0.5%).

Temperature treatment and family had a significant effect on the proportion of plants that became infected (Table 2). There was no significant temperature*family effect (Deviance =

296 20.434, df = 12, p=0.466). Infection was highest in the control treatment (constant 24 °C) and
297 showed a general pattern of decline with increasing temperatures (Figure 2). A one-way
298 ANOVA, followed by a Dunnett's test found that only the two hottest treatments (30 °C
299 immediate and four days delayed) were significantly different from the control (Figure 2,
300 Appendix S1: Table S3). The 30 °C immediate treatment had a 64% reduction in infection
301 compared to the control.
302 A total of 173 seedings died (9.6%) within 5 months of receiving the temperature
303 treatments. Seedling mortality was significantly affected by inoculation treatment and family, but
304 not by temperature (Table 3; Appendix S1: Figure S2). Mortality was higher in the inoculated
305 treatment (weighted average and 95% CI =12.3% +/- 0.016) than in the mock-inoculated
306 treatment (6.89% +/- 0.021). There were no significant interaction effects, as evidenced by the
307 loglikelihood comparison between a model with all pairwise interactions and a model with only
308 the main effects shown in Table 3 (Deviance = 21.314, df =31, p=0.903).
309
310 **Experiment 3: Effect of temperature on disease expression of infected plants in the field**
311 Temperature varied significantly over the course of the season, with significant heat
312 waves in July and August (Figure 3a). Plants flowered continuously over the season, with the
313 highest number in June and July (Figure 3b). Plants began to 'cure' and lose their infected
314 flowers in July, with symptomatic infections returning in September (Figure 3b).
315 Temperature, measured as the 14-day average, had a significant positive, linear effect on
316 the number of plants flowering (slope =8.693, se=1.187, df = 25, t=7.326, p<0.0001, Figure 3c).
317 Temperature had a non-linear effect on disease expression. Expression was not strongly
318 impacted by increasing temperatures up to a threshold close to 27°C, after which increasing

temperature led to a rapid loss of disease symptoms (Figure 3d). This cutoff value was confirmed by the two-line linear model, which was optimized at a tipping point between 26.5-27.2°C (Appendix S1: Figure S3, Table S4).

Discussion

Our results show that temperatures greater than 30 °C, an ecologically relevant temperature for *S. latifolia,* can strongly limit the germination, growth, and conjugation of the fungus *in vitro*, reduce infection success on seedlings, and result in temporary curing of infected adult hosts. Moreover, host survival and flowering are not impeded at 30 °C, indicating a thermal mismatch in the host's favor. This low heat tolerance of *Microbotryum* could help to explain why the disease is more prevalent on *S. latifolia* at higher elevations and latitudes where the climate is cooler (Abbate and Antonovics 2014, Bruns et al. 2018, Hood et al. 2010). Our results also suggest that increasing global temperatures could limit the future distribution of the disease.

Thermal tolerance of pathogen and host

From our *in vitro* experiment we found that three pathogen traits critical to infection (germination, growth, and conjugation) had a non-linear relationship with temperature, similar to other pathogens in plants and animals (Mboup et al. 2012, Mordecai et al. 2017, Scala et al. 2019, Ragonese et al. 2024). All three traits were strongly suppressed at temperatures approaching 30 °C. However, conjugation had the lowest heat tolerance, with an upper threshold of 25 °C, suggesting this may be the temperature-limiting trait for infection.

Our experiments on live plants indicated that this observed *in vitro* heat sensitivity of the pathogen can have important consequences for infection success and transmission *in vivo*. High

342 temperatures reduced the probability that exposed seedlings would become infected, in some
343 cases by as much as 64%. In both the 26 and 30 °C heat treatments, the reduction in infection
344 tended to be greater for seedlings that were heat-treated immediately compared to four days
345 delayed, indicating that the time window following exposure during which high temperatures can
346 prevent infection is limited. However, at 30 °C there was still a significant reduction in infection
347 even in the delayed heat treatment. Schäfer et al. (2010) observed infection structures on the leaf
348 surface four days post-inoculation and visualized the fungus in the intercellular spaces within the
349 plant 8 days post-inoculation, but there could be variation in this timing. Thus, it is unclear
350 whether the reduction in infection in the delayed 30 °C treatment was due to reduced survival
351 and conjugation on the leaf surface or reduced ability to infect and survive within the plant.
352 Perhaps most critically, we found that 2-week periods of high temperature (>27 °C)
353 temporarily cured infected adult plants in the field, enabling hosts to produce healthy flowers and
354 reducing the total duration of sporulation and transmission. The symptom-curing effects and the
355 new infection suppression effects of heat are likely to have compounded effect on transmission
356 in the field, with high heat leading to both a reduction in the number of infectious spores landing
357 on seedlings and a reduced infection rate for the spores that are produced.
358 While we found a strong suppressive effect of heat on the anther-smut pathogen, the plant
359 host was much more tolerant of high temperatures. We found no significant effect of temperature
360 on seedling mortality in the growth chamber, and heat had a strong positive effect on host
361 flowering in the field. These results indicate a mismatch in the thermal tolerance of pathogen and
362 host (Cohen 2017), with low heat tolerance in the pathogen but a more heat-tolerant host. Taken
363 together, our findings indicate that global warming could generate ‘thermal refugia’ (Gsell et al.

364 2023), regions of the host range where the warm temperatures significantly reduce transmission
365 rates, or even extirpate the disease locally, releasing the host population from pathogen pressure.
366 Our study adds to a growing body of work that shows a high diversity of thermal
367 tolerances among pathogens. For many pathogens, especially human vector-borne pathogens
368 (Mordecai et al. 2017), aquatic pathogens (Ward et al. 2007, Shocket et al. 2018), and well-
369 studied agricultural pathogens (Rossi et al. 2001, Bebber et al. 2013, Chaloner et al. 2021),
370 transmission is favored by warmer temperatures, leading to strong concerns that global warming
371 will exacerbate the threats of disease (Tracy et al. 2019, Chaloner et al. 2021, Stukenbrock and
372 Gurr 2023). In a zooplankton-fungal disease, warmer temperature have been experimentally
373 shown to increase transmission (Shocket et al. 2018). Among pathogens of natural plant
374 populations, manipulative warming experiments in alpine meadows have been shown to increase
375 the overall level of foliar pathogens in the community (Liu et al. 2019). Additionally, warmer
376 winters have been linked to increased survival of powdery mildew pathogens (Penczykowski et
377 al. 2015).
378 In contrast our results show that not all diseases are likely to increase with warming
379 temperatures. Similar heat sensitivity has also been reported for a few other pathogens such as a
380 protozoan parasites of monarch butterflies, where infection probability declines at higher
381 temperatures, however the host also suffers at higher temperatures (Ragonese et al. 2024)..
382 Among fungal pathogens of wild plants heat sensitivity has also been suggested by long term
383 observational studies: For example, Zhan et al. (2018) showed the local extinction rate of the rust
384 fungus *Triphragmium ulmariae* within a Swedish metapopulation of its host, *Filipendula*
385 *ulmaria* has increased with warming over a 26-year period. Likewise, Dudney et al. (2021) used
386 a 20-year data set to show that white pine blister rust (caused by the fungus *Cronartium ribicola*)

has moved up to higher, cooler elevations and been extirpated lower elevations in the Sierra Nevada. Our study contributes to these findings by showing that multiple components of pathogen life history are inhibited by high temperatures.

Ecological and evolutionary consequences

Our results indicate that climate change could strongly affect the seasonality of anther smut transmission. Longer and more intense periods of summer heat could limit transmission to shorter peaks in the spring and fall, as infected plants cure during the heat of summer. This shorter seasonal duration of transmission could lower prevalence and potentially drive local pathogen extinction. Alternatively, shorter and warmer winters could increase the suitable periods for flowering and sporulation in the spring and fall, offsetting the loss of summer transmission. Continued phenological measurements of both the host and pathogen will be critical to assessing these outcomes.

Increasing global temperatures could also affect the geographical distribution of the pathogen, driving a range shift to cooler higher latitude and higher elevation climates. Such a shift has been observed in another natural fungal disease, white pine blister rust. Dudney et al. (2021) found that, from 1995 to 2017, white pine blister rust prevalence has increased at high elevations and decreased at low elevations. A similar range shift to higher elevations could also happen in the anther-smut hosts for which the host range extends into higher elevations than the pathogen range. However, in *S. latifolia*, Bruns et al. (2018) found that anther smut is already present at the upper host range limits in the Italian Alps. Therefore, the ability of *Microbotryum* to shift its range in response to climate change could be limited by the range of the host.

The temperature sensitivity of *Microbotryum* could also lead to evolutionary changes in both the host and pathogen which could potentially feedback to affect disease prevalence (Jiranek et al. 2023). Specifically, the ability of high temperatures to prevent new infections and to temporarily cure existing infections could reduce selection for host resistance by allowing infected, susceptible genotypes to successfully reproduce during the hot summer months. Given that smut resistance can be costly (Biere and Antonovics 1996), this could result in selection favoring more susceptible genotypes in hot climates, which could in turn allow disease to persist even as the warming reduces the duration of transmission. Furthermore, the ability of the pathogen to adapt to warmer temperatures is currently unknown, but evidence of thermal adaptation has been observed in other fungal plant pathogens (Mboup et al. 2012, Boixel et al. 2022).

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Conflict of interest statement

We have no conflicts of interests to declare.

Author contributions

DC, SS, and EB designed the experiments. DC carried out experiments 1 and 2. AK carried out experiment 3. DC and SS analyzed the data. DC and EB wrote the manuscript, with input from all authors.

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Table 1. The effect of temperature on *in vitro* life history traits of *M. lychnidis-dioicae* in Experiment 1 and their predicted thermal thresholds. In all cases temperature was fit as a smoothing parameter, in a GAM model. Germination rate was fit with a knots of 5, and colony growth and conjugation rate was fit with a knots of 6 (See Appendix S1: Figure S1 for details). Statistical tests are the result of a likelihood ratio test from a full model containing replicate and parametric predictors of strain and mating type (See Appendix S1: Figure S1 for details).

Life History Trait	estimated df	F	p-value	Deviance explained	Lower threshold	Optimum	Upper threshold
Germination rate	3.658	34.49	<0.0001	64.1%	4.5 °C	13.5 °C	> 30 °C
Colony Growth	4.915	270.90	<0.0001	73.3%	10.6 °C	20 °C	29.0 °C
Conjugation rate	4.615	142.29	<0.0001	75.2%	< 4 °C	10.5 °C	25.7 °C

Table 2: Summary of a quasibinomial GLM showing the effect of temperature-treatment, plant family, and replicate on seedling infection rate in Experiment 2.

Source	Df	Deviance	Residual Df	Residual deviance	p
Temperature Treatment	4	53.482	50	92.55	<0.0001
Plant Family	3	38.988	54	146.03	<0.0001
Replicate dish	2	16.327	57	177.42	0.0117

Table 3: Summary of a binomial GLM showing the effects of temperature, inoculation treatment, and plant family on seedling mortality in Experiment 2.

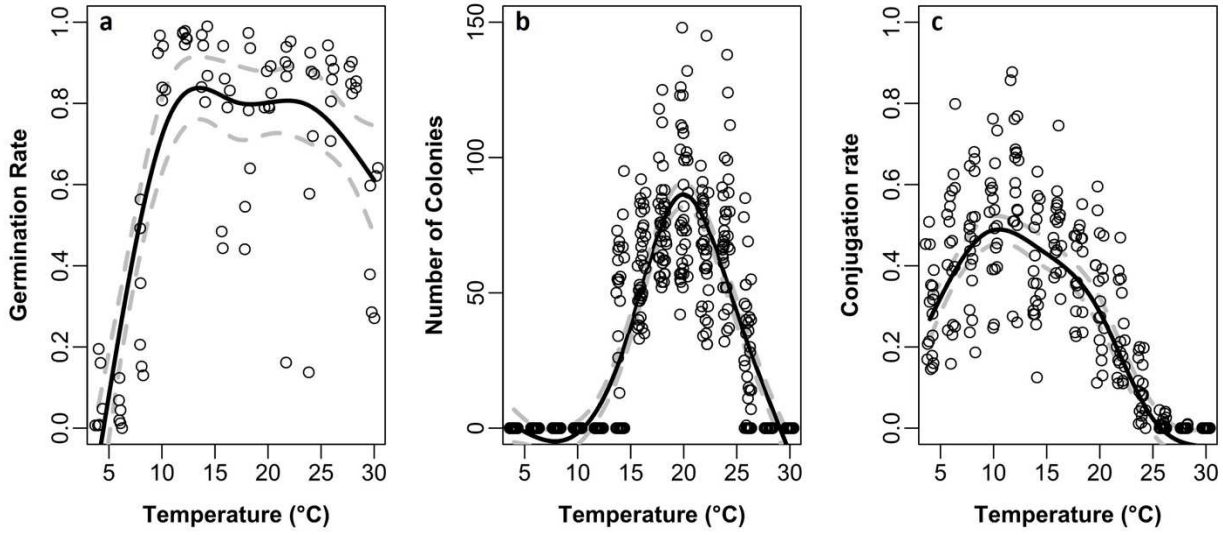
Source	Df	Deviance	Residual Df	Residual Deviance	p
Temperature treatment	4	6.91	71	79.46	0.14
Inoculation treatment	1	5.33	70	74.13	0.02
Family	3	8.56	67	65.57	0.04

Figure captions:

Figure 1: Relationship between temperature and *in vitro* spore traits measured in Experiment 1. (a) Germination rate was calculated as a proportion of germinated to total teliospores. (b) Growth was measured by the number of visible colonies that grew after one week following plating of approximately 100 sporidia. (c) Conjugation rate was calculated as a proportion of conjugating sporidia to total sporidia. GAM models (black lines) are plotted with the 95% prediction confidence interval (gray dotted lines).

Figure 2: Proportion of seedlings that became infected with Microbotryum across different temperature treatments in Experiment 2. Each shape/color represents the proportion of plants diseased for each of the four plant families tested. Treatment means and standard errors are predicted from the glm model. Significance of each treatment relative to the control was determined by a Dunnett test and denoted *P < 0.05 and ***P < 0.001 (See Appendix S1: Table S3).

Figure 3: Flowering and disease expression rates of infected Silene latifolia plants in the field (Experiment 3). A) Daily high temperature at the field site in Beltsville, MD from May to November 2021. Gray lines show day to day fluctuation, red line is a smoothed spline fit. B) Total number of flowering fully healthy cured flowering (blue dots and smoothed line) and partially or fully symptomatic plants (black dots and lines). C-D) Relationship between the average high temperature of the preceding 14 days and C) total flowering proportion, and D) proportion of flowering plants expressing anther-smut symptoms. Error bars are 95% confidence prediction intervals.


a
Germination Rate
0.0
0.2
0.4
0.6
0.8
1.0
5
10
15
20
25
30
Temperature (°C)
b
Number of Colonies
0
50
100
150
5
10
15
20
25
30
Temperature (°C)
c
Conjugation rate
0.0
0.2
0.4
0.6
0.8
1.0
5
10
15
20
25
30
Temperature (°C)

623

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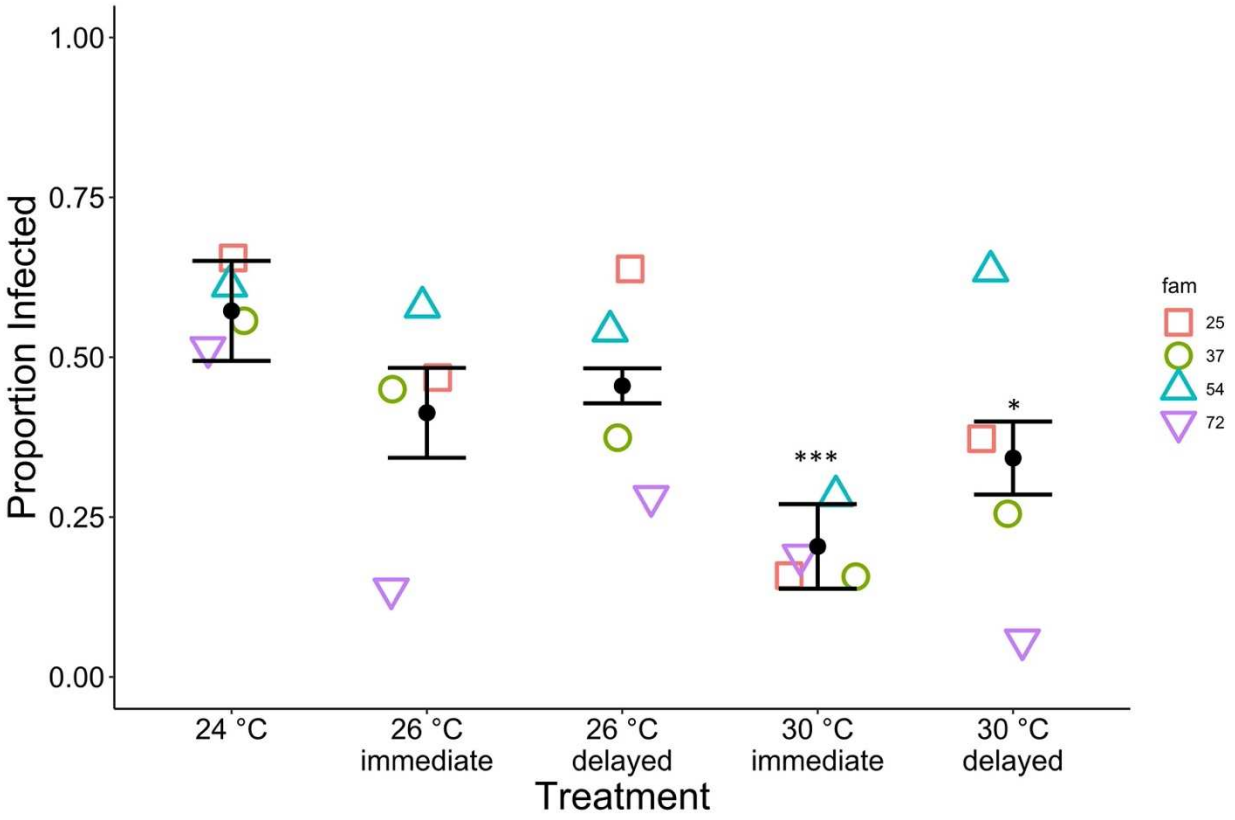
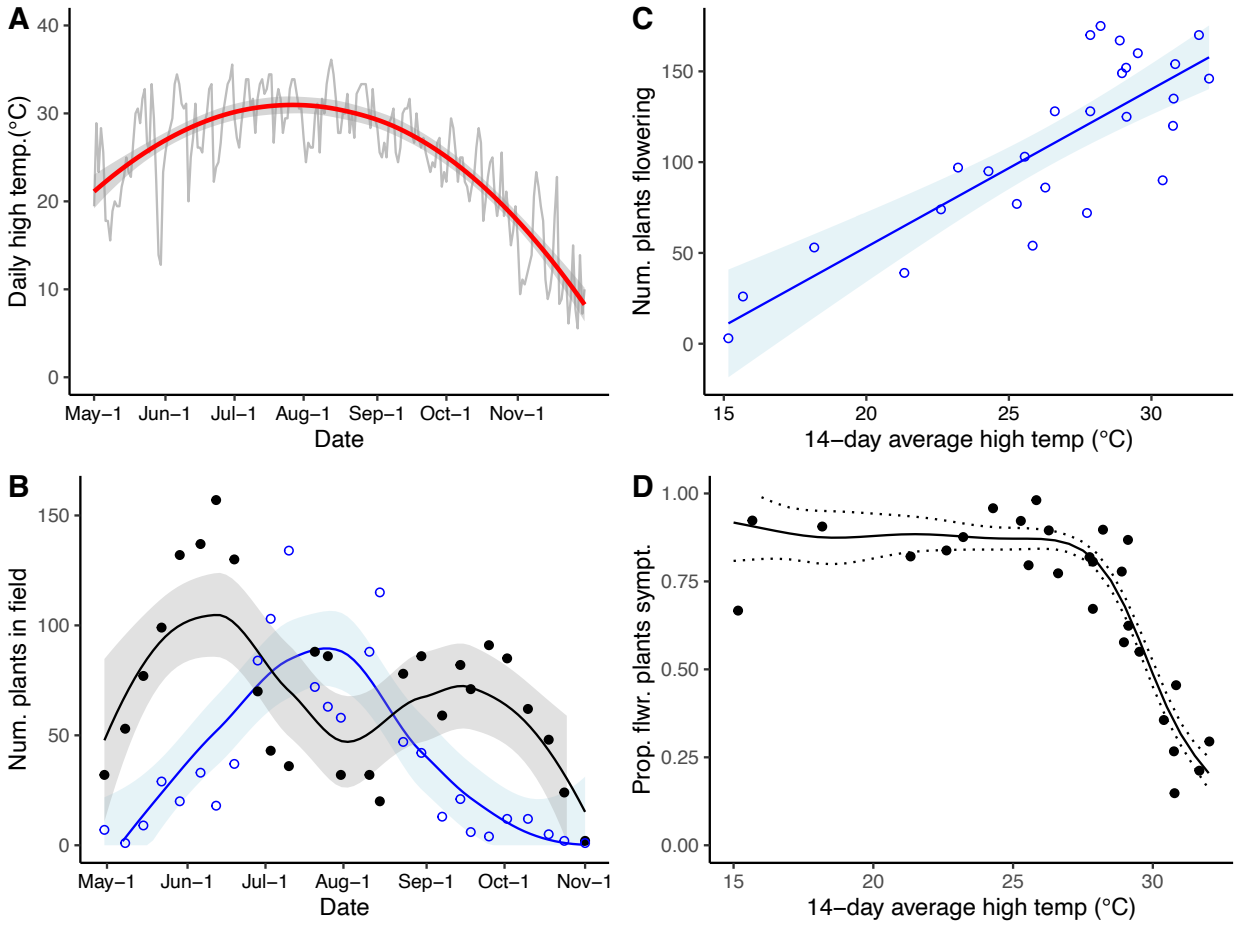

1.00
0.75
0.50
0.25
0.00
Proportion Infected
***
*
24 °C
26 °C immediate
26 °C delayed
30 °C immediate
30 °C delayed
Treatment
fam
25
37
54
72

626


A
Daily high temp.(°C)
40
30
20
10
0
May–1
Jun–1
Jul–1
Aug–1
Sep–1
Oct–1
Nov–1
Date
C
Num. plants flowering
150
100
50
0
15
20
25
30
14–day average high temp (°C)
B
Num. plants in field
150
100
50
0
May–1
Jun–1
Jul–1
Aug–1
Sep–1
Oct–1
Nov–1
Date
D
Prop. flwr. plants sympt.
1.00
0.75
0.50
0.25
0.00
15
20
25
30
14–day average high temp (°C)

627

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SUPPLEMENTAL MATERIAL: APPENDIX 1

High temperatures reduce growth, infection, and transmission of a naturally occurring fungal plant pathogen

By: Dalia V. Chen, Samuel P. Slowinski, Allyson K. Kido, Emily L. Bruns

Journal: Ecology- Article

Supplemental methods:

Dilution plating method for generating single mating type sporidial colonies.

Diploid teliospores were germinated for approximately 24 hours on 1% potato dextrose agar (PDA) plates until haploid sporidia formed. Plates were then washed with sterile DI water and dilution-plated onto a fresh PDA plate to generate single-sporidial colonies. Sporidial colonies were selected from each original teliospore culture and mating type was assessed through PCR with *M. lychnidis-dioicae* mating type specific primers (Xu et al. 2016). To amplify an a1 mating type specific sequence, the primers used were: MvSl_phero_a1_F: 5′-AGCC TGTGCACCGGATAG-3′ and MvSl_phero_a1_R: 5′-ACACCTCCAGCCTCAATAC TAACATCTC-3′. To amplify an a2 specific sequence, the primers used were: MvSl_phero_a2_F: 5′-AGCCGCCTCGAAGAGC-3′ and MvSl_phero_a2_R: 5′-AGTTCCGAAGGGCCACA-3′ (Xu et al. 2016). PCR product was run on a 1% agarose gel, and mating type was determined based on the presence or absence of visible bands. Mating type was confirmed with conjugation assays on 1% water agar plates (*Microbotryum* sporidia of oppositive making type form visible conjugation tubes on low nutrient media).

Table S1. Full summary of best-fit GAM models for *in vitro* pathogen traits. There were no significant interaction effects between temperature and either strain or mating type.

Germination rate

Term	Fit Type	df	Resid.df	F	p
Temperature	Smooth, fixed	3.658	4.000	34.49	< 0.0001
Replicate	Smooth, random	<0.0001	1.00	0	0.742

Colony growth

Term	Fit Type	df	Resid.df	F	p
Temperature	Smooth, fixed	4.9170	5.0	276.883	< 0.0001
Replicate	Smooth, random	0.7405	1.0	2.853	0.0502
Mating type	Parametric, fixed	1	NA	1.095	0.296
Strain	Parametric, fixed	2	NA	6.418	0.0018

Conjugation rate

Term	Fit Type	df	Resid.df	F	p
Temperature	Smooth, fixed	4.6198	5.0	144.25	< 0.0001
Replicate	Smooth, random	0.9649	1.0	27.52	< 0.0001
Mating type	Parametric, fixed	2	NA	2.673	0.0711

Table S2: Summary statistics showing deviance explained and Un-biased Risk Estimator (UBRE) values for binomial gam models of proportion spore producing plants given different temperature time blocks. Models with the lower UBRE scores are better fits to the data (Wood 2006). All models were fit with k=7, using package mgcv.

Temperature period (days)	**Deviance explained**	**UBRE**
7	67.8%	10.026
10	60.7%	12.244
12	73.2%	8.2168
14	**81.3%**	**5.5529**
16	81.2%	5.5166

Table S3: A) Summary of one-way ANOVA showing the effect of temperature-treatment on proportion of seedlings infected in Experiment 2. Each temp treatment includes 3 replicates of 4 host genotypes (n = 60 proportion values). B) results of a post-hoc Dunnett's test comparing to the control treatment.

A

Source	Sum Squares	Df	Mean Squares	F	p value
Temp Treatment	1.00	4	0.25	6.97	0.00106
Residuals	2.582	55	0.047		

B

Comparison	Difference	Adjusted p-value
Control vs 26°C immediate	-0.18	0.16
Control vs 26°C delayed	-0.13	0.42
Control vs 30°C immediate	-0.39	0.00025
Control vs 30°C delayed	-0.25	0.02

Table S4: Summary of two-line linear regression approach with variable temperature cutoffs. For each model we fit two separate linear regressions between 14-day average high temp and the proportion of flowering plants that show anther-smut symptoms. Overall residual error was minimized at 27°C.

Cutoff temp (°C)	df1	df2	resid.error.1	resid.error2	total error	
25	5	18	0.0942	0.1344	0.2286	
26	8	15	0.08826	0.1315	0.21976	
27	10	13	0.08888	0.1258	**0.21468**	*
28	13	10	0.09704	0.1283	0.22534	
29	16	7	0.1091	0.1423	0.2514	

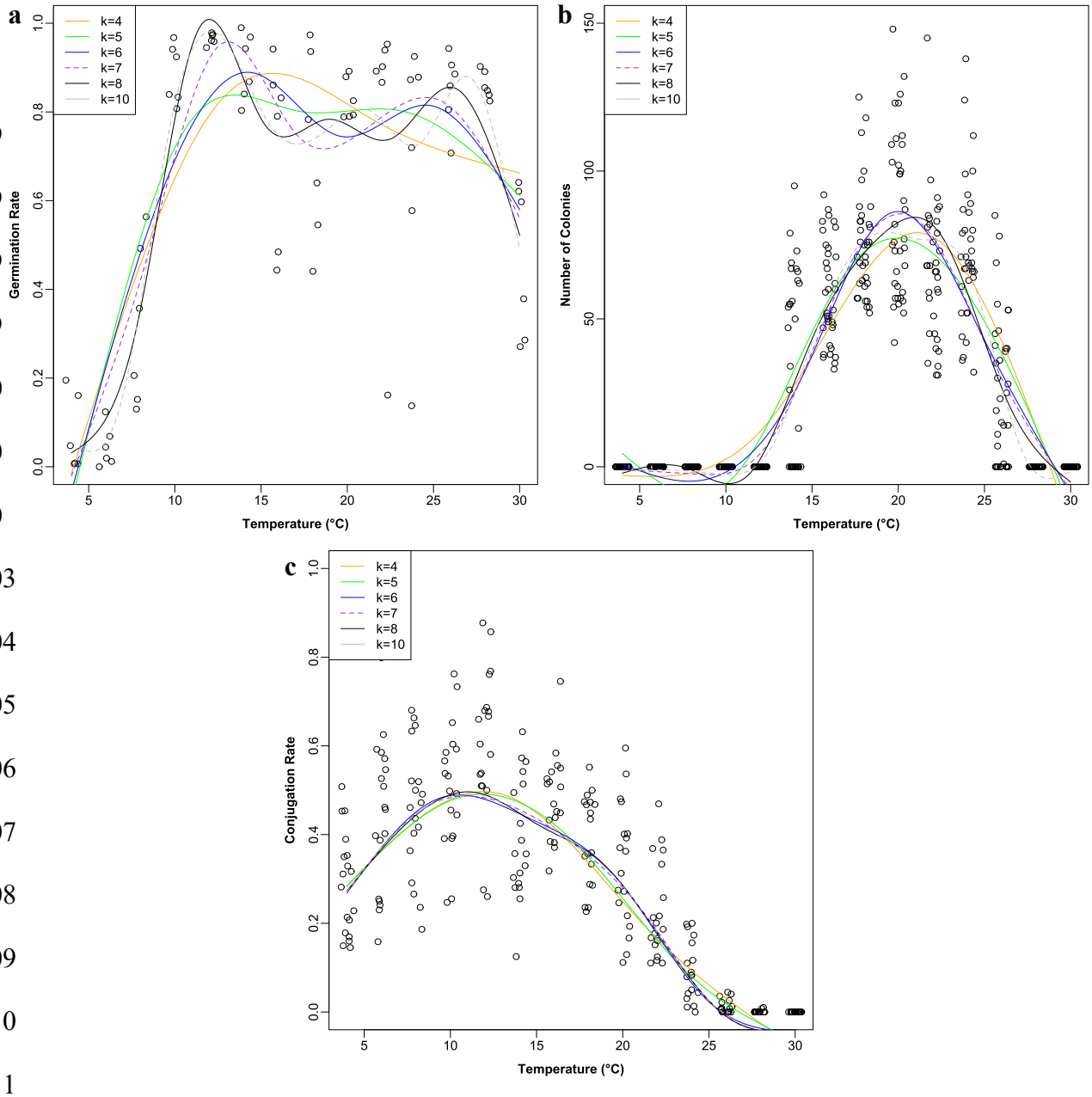
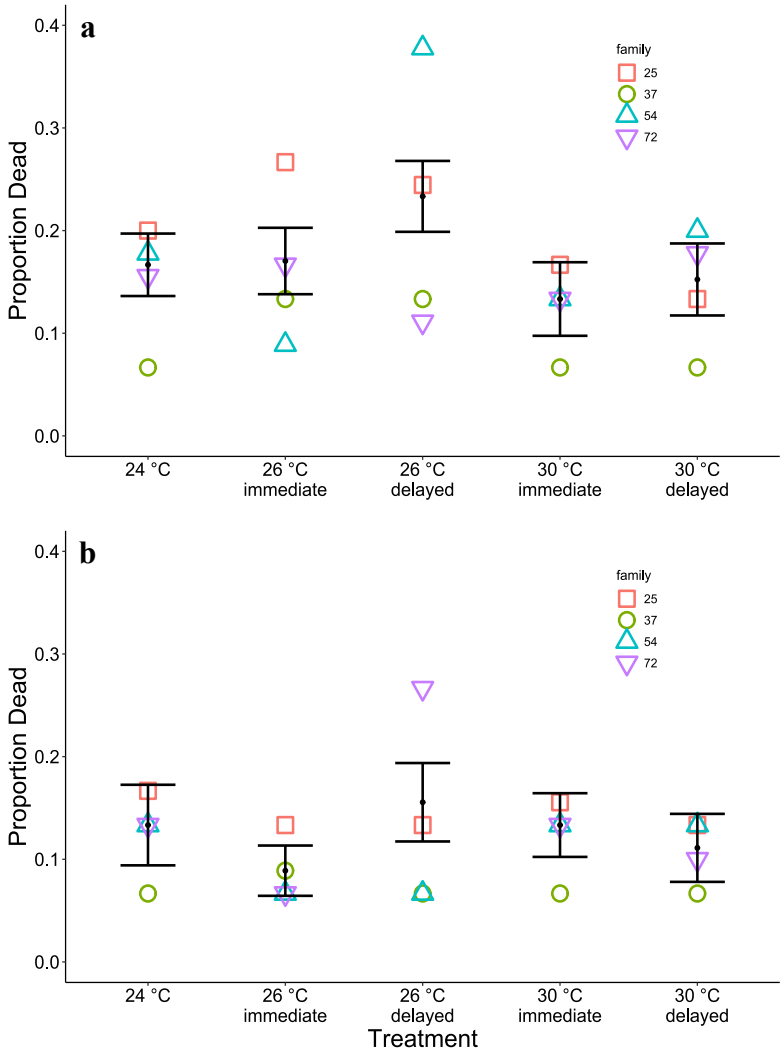


Figure S1: Generalized additive models with different number of knots fit to (a) germination rate, (b) number of colonies, and (c) conjugation rate data over a range of temperatures in Experiment 1. These graphs were used to choose the number of knots for the final model (k=5 for germination, k=6 for growth and conjugation). The black dots are the raw germination rate data, and each line is a generalized additive model fit using a different number of knots. Estimates of thermal maxima were generally robust to the choice of knots.



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721 **Figure S2:** Proportion of dead seedlings from each temperature and time treatment in

722 Experiment 2. (a) inoculated with a pathogen solution. (b) mock-inoculated with water. Each

723 shape/color represents the proportion of plants dead for each of the four plant families tested.

724 The mean proportion is represented by the black dot. Error bars are the standard error of the

725 mean.

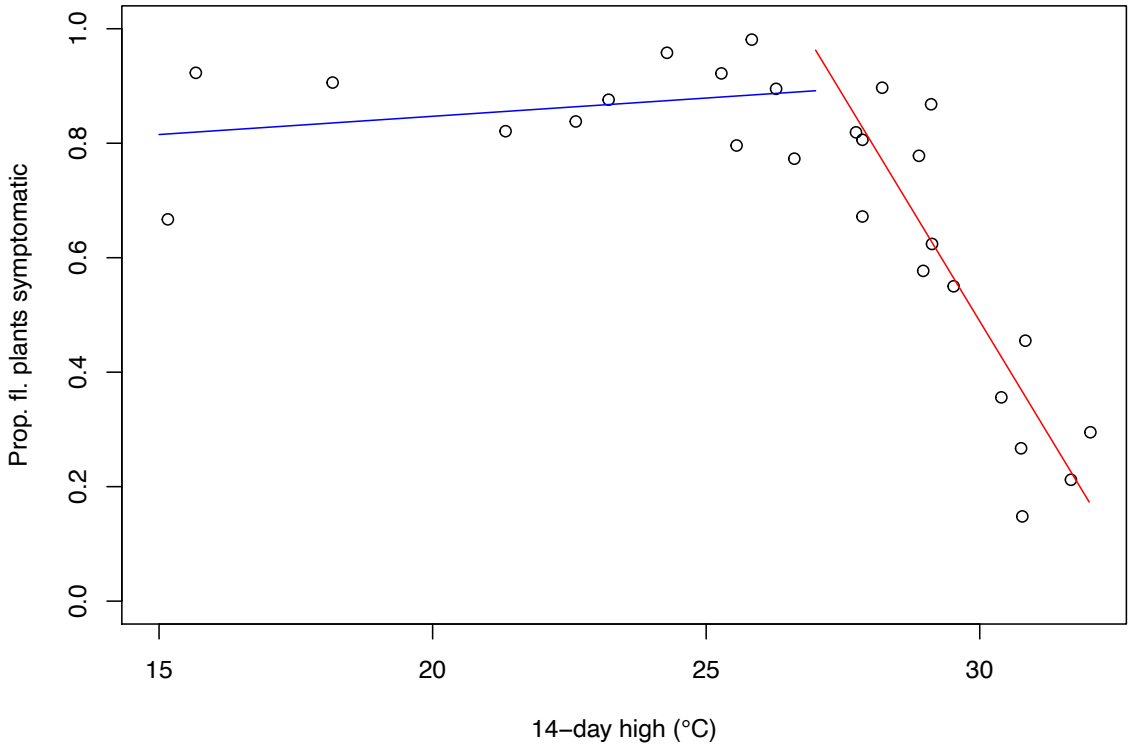

Figure S3: Best fit two-line model between average 14-day high temp and disease expression. The cutoff temperature here is 27°C F. The slope of the first, blue line is not significantly different from zero (slope=0.0064, df=10, se=0.0066, t=0.972 , p=0.3539), but the second red line is highly significant (slope = -0.15788, df=13, se= 0.0238, t=-6.624, p<0.0001).

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