

RESEARCH PAPERS

Effects of punctuated heat stress on the grapevine powdery mildew pathogen, *Erysiphe necator*

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Summary. Grapevine powdery mildew, caused by the ascomycete *Erysiphe necator*, is a major threat to grapes worldwide. Despite its global impact on grape production, *E. necator* is sensitive to adverse environmental conditions, such as excess heat, free water and UV radiation. Using detached leaf co-culture assays, 3-d-old single colonies of *E. necator* were exposed to 1, 2 or 3 consecutive d of punctuated heat stress. While there was a consistent decrease in colony growth after a single heating event, there were little to no significant effects from subsequent heating events. Similar effects were observed on the latent period of the pathogen, with a large initial effect from the first heat treatment and small marginal effects from subsequent heat treatments. *Erysiphe necator* colonies growing on live pot-grown plants were affected similarly by consecutive heat stress events. These data suggest that *E. necator* is more adaptable to environmental stress than previously recognized.

Key words: radial growth, temperature sensitivity, *Uncinula*, viticulture.

Introduction

While many fungi spend their lives embedded within substrates, epiphytic fungi are exposed to the rapidly changing environmental conditions of leaf surfaces and must tolerate stressful conditions to be successful colonizers. Conditions on the surface of a plant can fluctuate rapidly throughout a single day, with changes in temperature, relative humidity, radiation exposure and surface wetness all possible (Andrews and Harris, 2000; Carroll and Wilcox, 2003; Lindow and Brandl, 2003; Austin and Wilcox, 2012). Plant pathogenic fungi can often escape these stresses by growing endophytically, either intra- or intercellularly. However, the ascomycete fungal plant pathogens commonly known as the powdery mildews have an almost completely epiphytic growth habit, extracting water and nutrients using intra-cellular haustoria. The hyaline mycelia of pow-

dery mildews renders them susceptible to extreme environmental conditions (Willoquet *et al.*, 1996) with the result that these pathogens often grow best under mild temperature and lighting conditions. Their ability to acclimate and adapt to environmental stress is crucial to their survival.

The causal agents of powdery mildews are obligate plant pathogens (Glawe, 2008), thought to affect over 10,000 angiosperm host species, and are some of the most commonly encountered plant pathogens in natural and agricultural environments. Powdery mildews can regularly cause large economic losses in agriculture (Jarvis *et al.*, 2002). Grapevine powdery mildew, caused by *Erysiphe necator* (Schwein.), affects all *Vitis vinifera* grapevines grown in California, as well as several wild relatives of *Vitis* (Frenkel *et al.*, 2010; Cadle-Davidson *et al.*, 2011). Disease begins in the early spring with the release of ascospores from overwintering chasmothecia trapped in the bark of grapevines or perennation of mycelia in dormant buds (Gubler *et al.*, 1999; Rumbolz and Gubler, 2005). Rapid proliferation of asexual conidia on susceptible leaves can lead to epidemics if the pathogen

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is left unchecked. Disease can be controlled through the careful use of fungicides. Many growers use fungicides as a form of crop insurance (Horowitz and Lichtenberg, 1993), as the potential loss or crop damage from disease is often greater than the cost of any excess fungicide applications. However, such risk-averse behaviour can lead to over-application of fungicides if disease pressure is low or if the host is less susceptible. In order for growers to balance the risk of crop loss due to disease with the ecological impact of pesticide applications, better information about the true risk of disease is often needed.

Several epidemiological models have been created to predict disease pressure in an effort to reduce or optimize fungicide use (Sall, 1980; Chellemi and Marois, 1991; Gubler *et al.*, 1999; Calon nec *et al.*, 2008; Carisse *et al.*, 2009; Caffi *et al.*, 2011). Early studies found that grapevine powdery mildew grows well in mild conditions, and is capable of rapid germination, infection and growth between 21 and 30°C (Delp, 1954; Ypema and Gubler, 1997). Temperatures above 30°C are detrimental to growth, and are lethal or sub-lethal depending on exposure time. More recently, Calon nec *et al.* (2008) modeled the optimal infection of *E. necator* on grapevine as 22°C, with the minimum and maximum cardinal temperatures as 5 and 33°C, respectively. However, a recent study has shown that *E. necator* is capable of surviving long durations of high temperatures, including over 20 h of 36°C in detached leaf cultures (Peduto-Hand *et al.*, 2013). This suggests that the pathogen is able to colonize and grow under conditions that were previously considered to be lethal.

Many of these controlled environment studies have assessed the effects of a single, possibly continuous exposure to an extreme environmental condition on the germination, infection and growth of *E. necator*. These studies bring valuable insight and information for epidemiological models. However, in natural settings lethal and sub-lethal conditions are often punctuated by durations of tolerable or optimal conditions. This is exemplified by the many hot and dry grape growing regions of the world, such as some regions in Australia, Italy, South Africa, Spain and California. The San Joaquin Valley of California grew approximately two-thirds of the total US production of grapes in 2010 (CDFA, 2010). This important region often experiences consecutive days with temperatures considered to be detrimental to the pathogen ($\geq 30^\circ\text{C}$). Few studies have examined the

effect of multiple punctuated stress exposures on fungal growth and physiology.

In the present study we examined the effects of punctuated consecutive exposures of heat stress on the growth and latent period of *E. necator*. We treated 3-d-old colonies of *E. necator* on detached leaf plates to consecutive brief exposures of lethal and sub-lethal temperatures. Established colonies were chosen because conidia and young colonies often die when exposed to extreme heat conditions, even for short durations (Backup, 2009). We hypothesized that punctuated consecutive exposures would have a synergistic detrimental effect on the fungus, and that colonies exposed to consecutive heating events would cause an even greater delay in growth and latent period when compared with untreated or single-treated colonies. A similar experiment on live, potted plants was performed in a growth chamber to examine the robustness of the detached leaf assay results.

Materials and methods

Plant material

Grapevines (*Vitis vinifera*) of the highly susceptible cultivar Carignane were grown in a glasshouse in 3.8 L capacity pots containing commercial potting soil. The glasshouse was maintained at 30–40°C and had early-morning applications of vaporized sulphur to reduce the risk of powdery mildew disease epidemics. The grape plants were largely unaffected by the heat, and grew vigorously throughout the study. Continuous growth was obtained by frequent pruning, supplemental heat and light during the winter, and daily applications of dilute Hoagland's solution (Peduto-Hand *et al.*, 2013). The glasshouse was frequently scouted for diseases and pests. Unblemished, disease-free young leaves were harvested with petioles intact for use in detached leaf cultures. The third, fourth and fifth leaf from shoot growing tips were used to ensure maximum susceptibility and reduce the effects of ontogenic resistance (Doster and Schnathorst, 1985).

Detached leaf plates

Leaves were first washed in de-ionized water to remove any remaining sulphur or debris. Leaves were then surface sterilized in 0.6% sodium hypochlorite for 30 s and gently agitated to remove air

bubbles trapped in the abaxial trichomes. Leaves were then rinsed twice in de-ionized water, and allowed to dry on paper towels in a laminar flow hood. Petioles were cut to 1 cm using a sterile scalpel. Leaves were then immersed in cooled, liquid 1.2% water agar (Fisher Scientific Laboratory Grade Agar) with the abaxial side down, and the agar was allowed to cool and solidify in a sterile laminar flow hood. This immersion in water agar allowed for leaves to remain green for up to a month (Peduto-Hand *et al.*, 2013). After the water agar solidified, detached-leaf plates were placed in a sterile plastic crisper for 24 h to allow any remaining sodium hypochlorite to dissipate. Leaves were dried every 3 d using a laminar flow hood to reduce moisture and saprophyte contamination.

Mixed inoculum collection and maintenance

A mixed collection of five *E. necator* isolates, each originating from single conidia, were maintained on live, potted *V. vinifera* cv. Carignane plants in a growth chamber. Two isolates were collected from Davis, CA, the third was collected from Lodi, CA, the fourth from Clarksburg, CA, and the fifth was from Round Mountain, CA (Figure 1). The isolates from this collection came from different environments. The average number of growing degree days (Winkler (1965) degree day scale) for grapes in these areas from 2008–2012 were: 2066 in Davis, 2025 in Clarksburg, 1985 in Lodi, 1340 in Round Mountain, and 851 in Monterey. Isolates were randomly mixed together in the growth chamber to simulate natural inoculum. Plants were maintained at 25°C and irrigated with dilute Hoagland's solution. Plants were rotated into the chamber to maintain an actively growing population of *E. necator*. Leaves with young, actively growing 14 to 21 d-old colonies were randomly selected and removed and placed with their cut petioles inserted into a sealed water agar plate. At least five infected leaves were used as an inoculum source during each experiment. The random mixture of inoculum and random selection of inoculum-covered leaves helped to account for any variation in aggressiveness between the different isolates.

Mixed inoculum study

Detached leaf plates were each inoculated at several sites on the leaf using a single chain of conidia

at each inoculation site. A single chain of conidia was transferred from inoculum bearing leaves using a sterile eyelash affixed to a toothpick. Inoculation sites on the detached leaf plates were marked using a fine-tipped marker. Conidia from the inoculum leaves were dusted onto a glass microscope slide and placed in a moist chamber. Conidium germination rate was measured 24 h after inoculation on the glass slides. The inoculum leaves and the detached leaf plates were observed under stereomicroscopes during the infection to ensure the successful transfer of single chains of conidia. After all experimental detached leaves were inoculated, detached leaf plates were placed into a sterile plastic crisper and left at room temperature (22°C) for 3 d to allow the powdery mildew to colonize and establish. At 3 d post inoculation, all colonies on detached leaf plates were measured for colony diameter using a digital caliper under a Leica MZ9.5 stereomicroscope (Leica Microsystems) at 20× magnification, measuring the greatest colony diameter. Colonies that had not germinated or established after 3 d days were excluded from the study. After all colonies were measured, leaves were separated randomly into four treatment groups: a control group and three treatment groups of, respectively, one heat exposure, two heat exposures, and three heat exposures. Three days post inoculation, the control and experimental groups were measured for colony diameter and plates from all three experimental groups were randomly placed into plastic bags and exposed to 1 h hour of heat at 31, 33, 35, 37 or 39°C in a forced-air incubator (Thermo Fisher Scientific). After heating, experimental plates were cooled to room temperature in a laminar flow hood and randomly stored in a sterile crisper box. The control group was maintained at room temperature (22°C) and was never exposed to heating. At 4 d days post inoculation, the two heat exposure and three heat exposure experimental groups were again placed randomly into plastic bags and exposed to 1 h of heat treatment, cooled in a laminar flow hood and placed back into the sterile crispers. At 5 d post inoculation, all colonies were measured for colony diameter and each plate was assessed for latent period. After measurement, plates from the three heat exposure experimental group were placed randomly into plastic bags, exposed to 1 h of heat, cooled in a laminar flow hood and returned to a sterile crisper with the other plates. All heat treatments occurred 24 h apart. All plates were measured for colony diame-

ter on 3, 5, 7, and 11 d post inoculation, and all measurements occurred before heat treatments. All plates were monitored for the end of the latent period on 5, 6, 7, 8, 9, 10 and 11 d post inoculation. No colonies sporulated before 5 d post inoculation. All experiments included at least ten plates per treatment, and all experiments were repeated twice. A minimum of 160 colonies were measured over the time course for all treatments. Powdery mildew colonies overtaken by saprophytic fungi were not measured. Colony diameter was measured along the widest axis because powdery mildew microcolonies often grow laterally in the early stages of infection, and only begin expanding radially in later stages. Latent period was measured as the date that the first colony on a plate developed conidia.

Individual inoculum study

To test whether the effects of heat stress on colony growth and latent period were similar in different isolates, detached leaf co-culture assay tests were completed with three geographically distinct single-spore isolates. Two isolates were obtained from ascospores released from chasmothecia collected from Monterey, CA and Round Mountain, CA. The third single-spore isolate was collected from conidia on leaves in a vineyard in Clarksburg, CA. Isolates were maintained on detached leaf plates, were between 14 and 21 d old when used as inoculum, and were only opened in a sterile laminar flow hood. Care was taken to prevent cross contamination between leaf plates. Different isolates were inoculated randomly at different sites on the same detached leaf plate, rather than using separate detached leaf plates for each isolate. The random location of the inoculation site and the area that each isolate had to grow on allowed comparison of the effects of inoculum, without any cross-effect between different colonies. A separate transfer eyelash was used for each inoculum strain, and eyelashes were sterilized using 95% ethanol and blotted dry after every experiment. The number of leaf plates used in each experiment was increased to a minimum of 20 leaf plates per treatment.

In planta study

To confirm the detached leaf assay results, a similar experiment was carried out by infecting live

plants in a growth chamber. Rooted green 'Carignane' cuttings were grown for 2 months in 10 cm square pots filled with commercial potting soil. Plants were trained to metal stakes and separated from one another by empty pots in the tray. Plants were maintained in a growth chamber (Convicon, Winnipeg, Canada) at 22°C on a 12 h light/12 h dark period. Plants were irrigated with dilute Hogland's solution. Plants from the different treatments were randomly distributed throughout the chamber. The conidia of each of the three single-spore isolates were suspended in 0.05% Tween 20 (Acros Organics) and diluted to a final concentration of 1000 conidia per mL. Conidium concentrations were confirmed using a haemocytometer (Hausser Scientific). Fifteen mL of each suspension were mixed together to a final volume of 45 mL and the final conidium concentration was confirmed as 1000 conidia per mL. The suspension was applied evenly to the cuttings in the growth chamber onto the upper surfaces of all leaves using an aerosol pressurized sprayer (Preval Sprayer). Two microscope slides were placed among the plants on floor level to test the evenness of the spray inoculation and to confirm the proportion of germinating spores. Colonies were allowed to grow for 3 d days at 22°C. At 3 d post inoculation, colonies were identified using a 16× magnification hand lens and measured using a digital caliper. The plant and leaf number that each colony measurement was taken from was noted and recorded. Three d post inoculation, control plants were removed from the growth chamber and placed on a benchtop and the chamber was heated rapidly to 37°C for 1 h during the light period. After heat treatment, the chamber rapidly returned to 22°C and control plants were returned to the chamber. At 4 d post inoculation, control and one exposure plants were again removed from the chamber for 1 h and replaced after the chamber had returned to 22°C. At 5 d post inoculation, colonies were measured and control, one exposure and two exposure plants were removed from the chamber during the heat treatment, and replaced after the chamber returned to 22°C. All heat treatments occurred 24 h apart. Colony diameter was measured on 3, 5, 7, 9 and 11 d post inoculation. No observations were made regarding latent period due to the confounding factor of increased wind within the growth chamber from the forced temperature-controlled air dislodging conidia.

Statistical analyses

All statistical analyses were performed using JMP v. 9.0 (SAS Institute). The results of the individual inoculum study were analyzed first to see whether isolate or the interaction of isolate and treatment (isolate \times treatment) had significant effects on colony diameter. Treatment was defined as both the number of heating exposures (0, 1, 2, or 3) and the temperature of the heat exposure (untreated control, 31, 33, 35, 37, or 39°C). The inoculum data were analyzed using a mixed-model approach, using plate number as a random effect and treatment, isolate, and isolate \times treatment as fixed effects. Data from each temperature group were analyzed separately using the mixed model. After first confirming that there were no differences between the growth patterns of different isolates, data from the mixed inoculum and individual inoculum study were combined. A linear regression line was fit through the colony growth data for each treatment within an experiment.

Latent period data were analyzed using a time-to-event (or survival analysis) approach. Latent period data were analyzed by temperature group using a Log-Rank test, and a chi-squared test was used to test for differences between groups. Log-Rank tests were also used in a pairwise fashion between treatments within a temperature group. This allowed for comparison of latent periods within each temperature group. A Bonferroni adjusted alpha ($\alpha=0.0083$) was used to protect the multiple comparisons from false positives, by dividing the standard alpha ($\alpha=0.05$) by the number of comparisons in each group (Bland and Altman, 1995).

For the *in planta* tests, data were analyzed using a mixed model using plant number and leaf number as random effects and treatment as a fixed effect. *In planta* colony diameter data were compared with 37°C detached leaf assay data using a mixed model approach by treatment, with experiment type (*in planta* and detached leaf assay) as a fixed effect, and leaf and plate number as a random effect. The two experiment types were compared using Student's test.

Results

Effect of inoculum

The isolates used in this study were similarly affected by consecutive heat stress events ($P>0.05$).

Isolates were collected from geographically and climatically diverse regions within California. There were no significant differences between the colony diameter growth of different isolates ($P>0.05$) and there was no significant interaction between isolate and treatment ($P>0.05$). There was also no significant difference between the data from both the mixed inoculum study and the individual inoculum study ($P>0.05$). There was a consistent significant difference between the treatment and control in all temperatures in both the mixed inoculum study and individual inoculum study ($P<0.0001$). There was no significant difference in germination rate of spores on glass slides between different isolates ($P>0.05$).

Effect on colony diameter

Colony diameter growth was negatively affected by heat stress. There was a consistent significant reduction in growth in colonies that had been heat-stressed once compared with the control (Table 1, Figure 1). However, subsequent heating events were not consistently associated with additional detrimental effects. While there were some additional detrimental effects at high temperatures from successive heat treatments, the effects of later heat treatments were not of the same magnitude as the effect from the initial heat treatment (Figure 1). There was a significant difference between colonies treated with one exposure at 37°C and colonies treated with two exposures (Figure 1d). A similar effect occurred at 39°C, where colonies treated with one exposure were significantly less affected than those treated with two or three exposures (Figure 1e). The linear regression showed that the growth rates of the untreated colonies were greater than the growth rates of any of the heat treated colonies.

Effect on latent period

Latent period was negatively affected by heat stress. The latent period was consistently delayed by a single heating event (Figure 2). However, subsequent heating events did not consistently elicit additional detrimental effects. Log-rank tests are used in survival analyses to assess whether there are significant differences between groups. All temperature treatments were analyzed using a Log-rank test which revealed differences between treatments in every temperature group ($P<0.0001$). Following

Table 1. Results of the linear regression of *Erysiphe necator* colony growth and the mean colony diameter from detached leaf assays and the *in planta* assay.

Treatment temperature (°C)	Number of exposures	Degrees of freedom	Intercept (mm) ± SEM	Growth rate (mm/day) ± SEM	Mean colony diameter (mm) ± SEM
Detached leaf assays					
31	Untreated	208	0.82 ± 0.14	0.45 ± 0.01	3.99 ± 0.07
31	1	196	1.25 ± 0.14	0.32 ± 0.02	3.54 ± 0.06
31	2	171	1.28 ± 0.14	0.29 ± 0.02	3.33 ± 0.06
31	3	246	1.25 ± 0.11	0.33 ± 0.02	3.49 ± 0.05
33	Untreated	225	0.12 ± 0.09	0.70 ± 0.01	5.03 ± 0.07
33	1	253	0.60 ± 0.10	0.55 ± 0.01	4.41 ± 0.06
33	2	195	0.74 ± 0.10	0.52 ± 0.01	4.39 ± 0.06
33	3	178	0.48 ± 0.09	0.53 ± 0.01	4.20 ± 0.06
35	Untreated	235	0.98 ± 0.11	0.75 ± 0.02	5.96 ± 0.08
35	1	197	1.60 ± 0.11	0.52 ± 0.02	5.04 ± 0.06
35	2	186	1.60 ± 0.11	0.47 ± 0.02	4.75 ± 0.06
35	3	204	1.56 ± 0.11	0.50 ± 0.02	4.85 ± 0.06
37	Untreated	252	0.29 ± 0.10	0.78 ± 0.01	5.50 ± 0.07
37	1	213	-0.04 ± 0.09	0.71 ± 0.01	4.79 ± 0.07
37	2	183	0.34 ± 0.10	0.61 ± 0.01	4.50 ± 0.06
37	3	224	-0.09 ± 0.09	0.69 ± 0.01	4.55 ± 0.06
39	Untreated	210	0.52 ± 0.09	0.69 ± 0.01	5.28 ± 0.06
39	1	202	0.51 ± 0.08	0.56 ± 0.01	4.36 ± 0.05
39	2	174	0.35 ± 0.09	0.54 ± 0.01	4.12 ± 0.06
39	3	161	0.58 ± 0.09	0.48 ± 0.01	3.87 ± 0.05
<i>In planta</i> assay					
37	Untreated	52	1.06 ± 0.26	0.66 ± 0.03	5.70 ± 0.15
37	1	78	0.89 ± 0.19	0.54 ± 0.02	4.70 ± 0.11
37	2	79	1.05 ± 0.20	0.54 ± 0.02	4.82 ± 0.11
37	3	72	0.95 ± 0.19	0.51 ± 0.03	4.51 ± 0.10

the overall test of a treatment effect, pairwise Log-rank tests were used to assess whether there were differences within a temperature group. There was

a consistent significant difference between the latent period of the control group and the experimental groups. Based on the median latent period (Figure

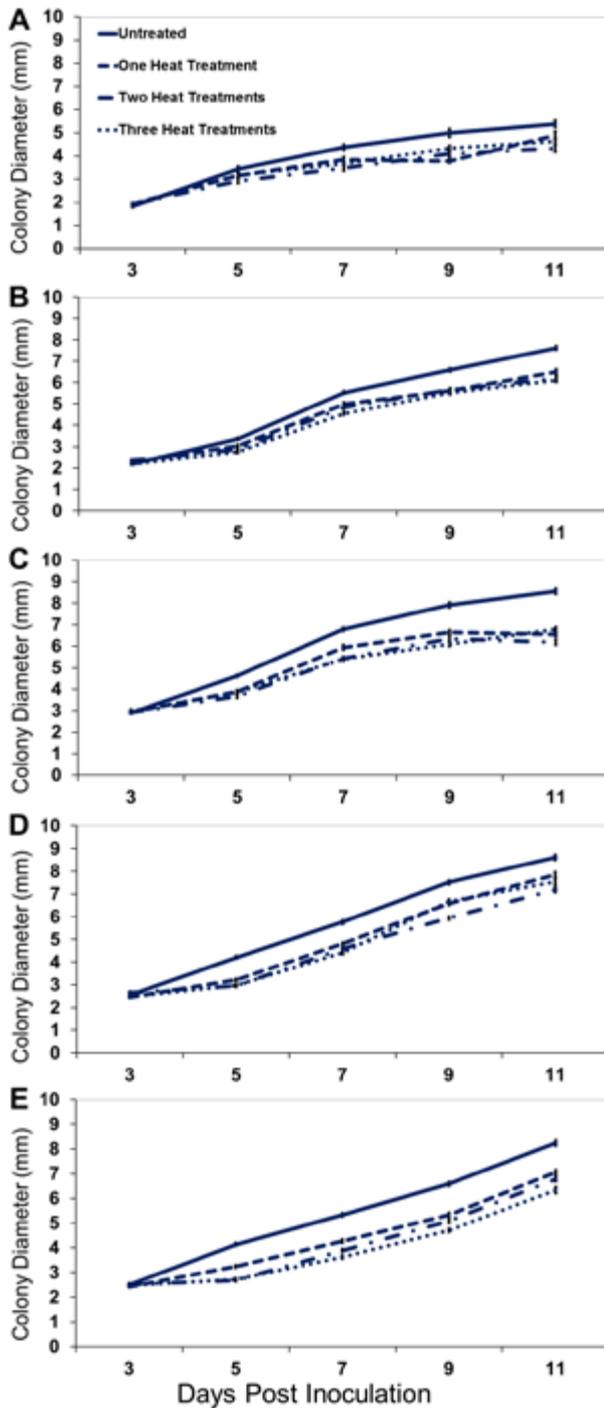


Figure 1. *Erysiphe necator* colony diameter growth for different temperature and heat exposure groups at A) 31°C, B) 33°C, C) 35°C, D) 37°C, and E) 39°C. Points represent true means \pm the standard error of the mean.

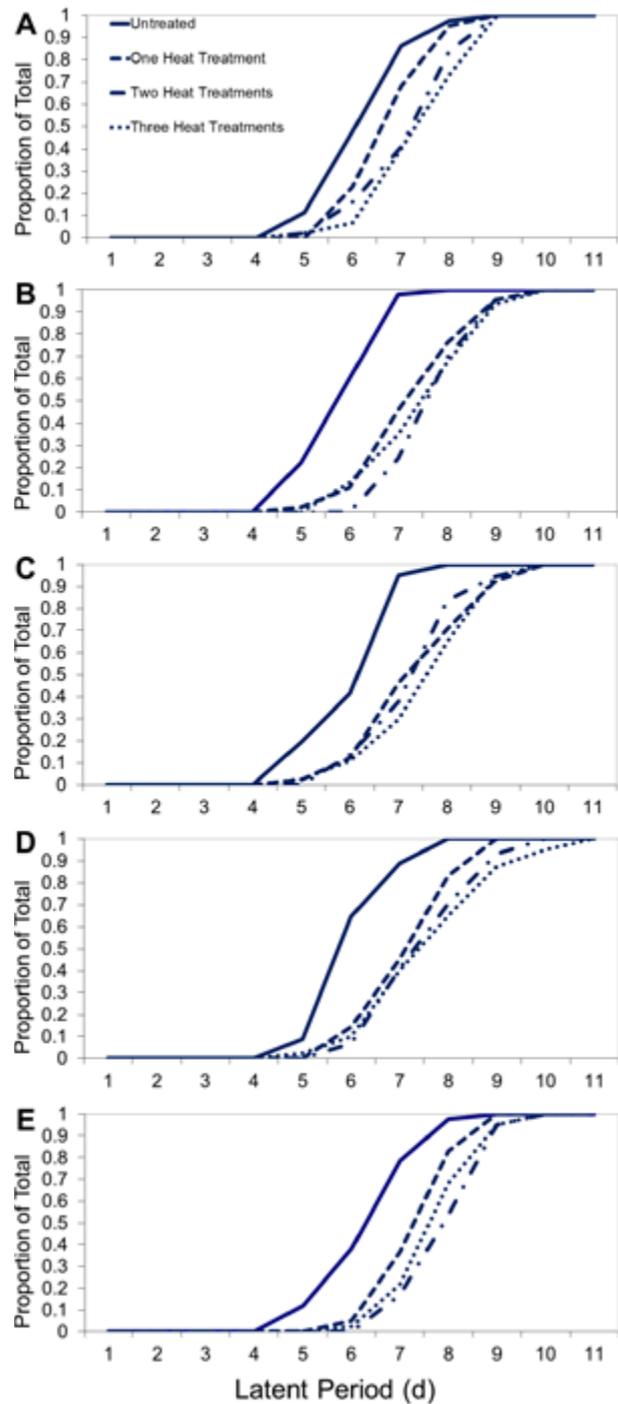


Figure 2. *Erysiphe necator* latent periods (d) for different temperature and heat exposure groups at A) 31°C, B) 33°C, C) 35°C, D) 37°C, and E) 39°C.

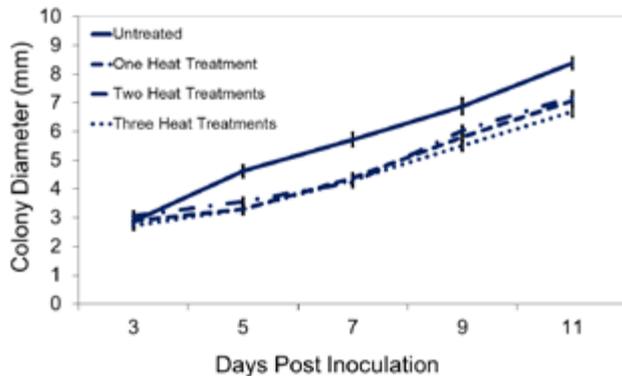


Figure 3. Colony diameter growth of *Erysiphe necator* on live plants for different heat exposure groups at 37°C. Points represent true means \pm the standard error of the mean.

compared by treatment using a mixed model approach. There was a significant difference between the *in planta* assay and the detached leaf assay in the two heat-treatment experimental group ($P=0.01$). There were no significant differences between the other experimental treatment groups or the control ($P>0.05$).

Discussion

After an initial heat event *E. necator* growth was relatively unaffected by additional heat stress events. While the first exposure to heat stress slowed the growth and extended the latent period of *E. necator*, the second and third exposures did not have consistent additional detrimental effects. These observations were consistent in both the detached leaf assays and the *in planta* assay and were also consistent across isolates collected from different geographic and climatic regions of California. The delay in growth and increase in latent period were exacerbated with increasing temperatures.

A comparison of the response to heat stress across different isolates from different geographic and climatic regions revealed that the observed phenomenon was not unique to isolates from hot regions. Single-spore California isolates were collected from cooler regions (e.g. - Monterey) and warmer regions (e.g. - Davis), and the effect of heat stress was consistent. Recent studies have shown that there is relatively low genetic diversity of Western US *Erysiphe necator*

populations compared with populations from the Eastern US (Brewer and Milgroom, 2010). It is possible that this reduced genetic diversity may underlie the low phenotypic diversity observed here, and that a study on a broader population would reveal more variation in acclimation to heat stress in *E. necator*.

A recent study (Peduto-Hand *et al.*, 2013) found that *E. necator* colonies could withstand single continuous exposures to extreme temperatures. While there were sub-lethal effects, such as increased incubation and latent periods and reduced conidium production, *E. necator* colonies were able to resume their life cycles after a heat stress event. Our study confirmed the ability of *E. necator* to resume its life cycle, even through repeated heat exposures. Peduto-Hand *et al.* (2013) were able to successfully transfer the laboratory results to a field-tested epidemiological model. Their field results confirmed that the pathogen is capable of withstanding more extreme environmental conditions than previously recognized.

Erysiphe necator is able to tolerate multiple exposures to high temperatures, with reduced marginal effects of repeated exposure. This reduction in detrimental effect is consistent with the phenomenon known as acclimation, where an individual organism can temporarily adjust to unfavorable conditions (Townsend *et al.*, 2009). Acclimation leads to a threshold of inhibitory effects from extreme environmental conditions. It is contrasted with adaptation, where a population of organisms permanently adjusts to new conditions over the course of several generations. Huey *et al.* (1999) found that while most cases of acclimation had a beneficial effect, some instances led to reduced fitness in the acclimated organisms. These detrimental effects were often caused by damage to the organism during the acclimation process. Beneficial acclimation most often occurred in extreme temperatures, with an organism risking reduced fitness against the probability of lethal conditions. Thus, while acclimation can be distinguished from adaptation essentially by considering whether the response is short- or long-term, the capacity for acclimation may itself be an adaptive strategy, in organisms subjected to uncertain environments.

While many studies of acclimation have been directed at ectotherms (Huey *et al.*, 1999), organisms that rely on environmental heat sources, relatively few have directly studied temperature acclimation in fungi (Lange and Green, 2005). This discrepancy may be due to the difficulty in separating the fungi from

their natural substrates unharmed. There has been a recent interest in the role of temperature acclimation of ectomycorrhizae and lichens because of their significant impact on ecosystem respiration (Lange and Green, 2005; Malcolm *et al.*, 2008). These studies have found that many species are able to acclimate to new conditions, adjusting their growth and respiration in higher temperatures. Acclimation in fungi can also lead to other ecological effects. Sharifi *et al.* (2007) found that an arbuscular mycorrhizal fungus that had been pre-treated with salt was able to confer salt tolerance to soybean, leading to increased uptake of nutrients and increased dry weight of the plant.

Recently, Moyer *et al.* (2010) found that exposing grape leaf tissue to cold temperatures prior to inoculation reduced *E. necator* colony area and increased colony mortality. Moyer *et al.* proposed that the exposure of grape leaf tissue to extreme temperatures either activated a temporary host defense response or made host tissue unsuitable for colonization. We expected an intensified reduction in growth and increased latent period in repeated heat treatments due to the combined effects of detrimental temperatures and a host defense response. However, our results did not show an increased detrimental effect from additional heat treatments. An ideal control to test the effect of host defense response would be to heat treat and measure colony diameter and latent period outside of the host. However, the obligate biotrophic nature of this fungus makes this experimental control impossible.

Acclimation to heat stress in *E. necator* can impact the epidemiology of the pathogen. Several epidemiological models aiming to predict disease intensity (Sall, 1980; Gubler *et al.*, 1999; Carisse *et al.*, 2009) assume that air temperatures greater than 35°C will completely inhibit infection, growth, and sporulation of *E. necator*. However, the observed acclimation of *E. necator* to consecutive days of heat stress would not be accounted for in these models. These models would associate consecutive days of high temperatures with reduced disease pressure and an inhibited pathogen. However, acclimation of *E. necator* would result in a more resilient and vigorous pathogen than predicted. This asymmetry could miss potential infection periods and lead to missed fungicide applications, resulting in damage to the crop.

While most studies on fungal growth rely on air temperature, there is a growing body of research that suggests that leaf surface temperatures may be very

different from air temperatures (Delp, 1954; Moyer *et al.*, 2010; Austin and Wilcox, 2012). Temperature and humidity near leaf surfaces can vary due to the effects of transpiration and wind speed (Gates, 1968). This disparity could lead to errors in estimating disease threat in epidemiological models. This study used air temperature to assess the effects of environmental conditions on leaves. A recent study (Peduto-Hand *et al.*, 2013) used thermocouples to compare the difference between air temperature and the temperature of leaves in detached leaf cultures. They found that the discrepancy between the two measurements was less than 1°C.

The obligate biotrophic nature of *E. necator* makes it difficult to conduct *in vitro* studies of the biochemical pathways that lead to stress responses. Compounds extracted from leaf-cultured tissue could easily come from either the host or the pathogen, or both. Cadle-Davidson *et al.* (2009) recently proposed a reliable method to extract RNA from *E. necator* without contamination from the host. This extraction method could be coupled with a multiplexed reverse transcription qPCR assay to study differential expression of heat-stress tolerance proteins. Future studies may explore gene expression of proteins associated with heat stress tolerance in other fungi, such as trehalose-6-phosphatase (Al-Bader *et al.*, 2010), calmodulin (Kraus *et al.*, 2005), or superoxide dismutase (Abrashov *et al.*, 2008). These proteins may be upregulated in heat-stressed *E. necator* colonies, leading to the observed acclimation.

Erysiphe necator is detrimentally affected by many different types of stress. Some forms of stress, such as extreme cold and heat, can occur for several consecutive days. Other forms of stress, like rainfall or fungicide applications, occur more sporadically. Acclimation and adaptation to stress is crucial to survival for *E. necator*. This study has demonstrated that *E. necator* is capable of acclimating to repeated acute heat stress events, even in temperatures considered to be detrimental to growth and sporulation. It is possible that this plasticity could extend to other forms of persistent or regular stress, such as rainfall or extended periods of cold. Acclimation to repeated environmental stress could also disrupt epidemiological models that attempt to predict disease pressure based on daily environmental conditions (Gubler *et al.*, 1999; Mahaffee *et al.*, 2003). Modeling lethal or sub-lethal conditions when a pathogen has acclimated to stress could lead to missed applications of

protective fungicides, causing valuable crop losses. Heat acclimation may significantly impact practical control of grapevine powdery mildew but is so far a little-studied area of mycology.

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