

Relative heat sensitivities and the potential for soil solarization to remediate nursery beds  
infested with *Phytophthora* spp.

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## **Relative heat sensitivities and the potential for soil solarization to remediate nursery beds infested with *Phytophthora* spp.**

### **Abstract**

Infestations of container nursery beds by *Phytophthora* spp. can be persistent and costly. One method of disinfestation that does not require the use of chemicals is soil solarization, which captures energy from the sun to heat soil and thermally inactivate target pathogens. In laboratory temperature gradient experiments, I investigated the thermal sensitivities of *P. ramorum*, *P. pini*, *P. chlamydospora*, and *P. gonapodyides* by subjecting inoculum samples to temperatures ranging from 30 – 40 °C. Field trials of soil solarization were conducted in July and August 2014 in San Rafael, California and Corvallis, Oregon. Leaf inoculum was buried at 0, 5, and 15 cm in California and Oregon solarization field trials. *P. pini* and *P. chlamydospora* were tested in both locations, however due to quarantine restrictions *P. ramorum* was only included in the California field experiment. In laboratory experiments estimated what temperature treatment would be effective in eradicating 99.9% of samples (LD<sub>99.9</sub>) which varied by species and inoculum type. With trials utilizing filter paper inoculum, *P. gonapodyides* was the most resilient to heat, with an estimated LD<sub>99.9</sub> of 42.55 °C. Trials in which rhododendron leaves served as an inoculum, *P. chlamydospora* was the most resilient to heat, with an estimated LD<sub>99.9</sub> of 47.66°C. Solarization for 2 or 4 weeks eliminated recovery of *Phytophthora* spp. from all depths in both locations, with the exception of *P. chlamydospora* at 15 cm in California, which was recovered during sampling at 2 and 4 weeks in this location. Estimated heat tolerances of the four *Phytophthora* spp. were different from one another, however because of the inconsistencies between trials and inoculum type it remains unclear which species are truly the most heat tolerant. Results from field trials of soil solarization indicate that this is a promising treatment for nursery beds in both Oregon and California infested with *Phytophthora* spp.

## Introduction

Oomycetes, commonly called water molds, are eukaryotic organisms in the kingdom Stramenopila. Although they were historically thought of as fungi due to morphological similarities, molecular phylogenetics have shown that the Oomycota are actually more closely related to brown algae and diatoms than to the organisms of kingdom Fungi (Baldauf 2003). Oomycetes, like many true fungi, are made up of filamentous hyphae; have absorptive nutrition, and produce asexual and sexual spores. They also possess several morphological characteristics that set them apart from the organisms in the kingdom Fungi. Unlike the true fungi, they have heterogametangial sexual reproduction involving the fertilization of oospheres by the nuclei of antheridia to produce oospores, their vegetative mycelia are diploid, their cell walls are composed of  $\beta$ -glucans and cellulose but not chitin, and they have motile biflagellate asexual spores, called zoospores (Webster and Weber 2007).

*Phytophthora* is a genus in the class Oomycota whose name is derived from the Greek words for plant and destruction. The first species of “plant destroyer” to be described was *Phytophthora infestans*, which was shown to be the causal agent of potato late blight by the German mycologist Anton de Bary in 1876 (Webster and Weber 2007). Late blight of potatoes resulted in the great Irish potato famine of the 1840’s leading to the loss of over one million Irish lives through starvation, and even more through emigration (Callaway 2013).

Another *Phytophthora* species is associated with a more recent disease commonly known as Sudden Oak Death (SOD), which has reached epidemic proportions in 14 coastal California counties and as far north as Curry County in Oregon. The disease is characterized by bleeding cankers on trunks of certain species of oak and tanoak trees (Rizzo et al. 2002). On other hosts, the pathogen causes foliar blight. The causal agent, *P. ramorum* Werres, de Cock & Man in’t

Veld was first described in 2001 in Germany and the Netherlands where it was shown to cause a twig blight on rhododendron and viburnum (Werres et al. 2001). *P. ramorum* has a wide host range, with 109 recognized known hosts(citation). In natural systems it causes extensive mortality in tanoak (*Notholithocarpus densiflorus*) and the red oaks (*Quercus* spp. section Lobatae) in the western United States, and Japanese larch (*Larix kaempferi*) in the United Kingdom (Grünwald et al 2008). Further genetic analysis has established that there are four known, distinct lineages of *P. ramorum*, and that this pathogen has made at least four global migrations (Grünwald et al. 2012; Van Poucke et al. 2012) .

The presence of *P. ramorum* in wildlands in California and Oregon is most likely the result of the pathogen being introduced through the nursery trade (Goss et al. 2009; Croucher et al. 2013). Unfortunately *P. ramorum* is not unique in its role as a *Phytophthora* pathogen introduced by international plant trade. Globally, other invasive examples in this genus resulting from the international trade of plants include *P. kernoviae*, *P. cinnamomi*, *P. alni*, and *P. quercina* (Brasier 2008). *P. ramorum* is a quarantined pathogen in the United States and regulations have been put in place in order to avoid its further spread (USDA APHIS, n.d.). For horticultural nurseries, the detection of *P. ramorum* on site can limit trade and thus has the potential to be very costly. A recent survey of four horticultural nurseries over a four year sampling period in Oregon found 28 different taxa of *Phytophthora*, which could be separated into ecological guilds based on their associated habitats (Parke et al. 2014). Similarly, field surveys investigating the presence of particular *Phytophthora* species in forests also indicate that this genus is rich in species and ecologically diverse (Hansen et al. 2012). The diversity of *Phytophthora* species range from foliar pathogens, to soilborne fine-root and canker pathogens,

to aquatic opportunists, facultative pathogens which are primarily isolated from streams (Hansen et al. 2012).

In commercial nurseries it is especially important that detection of known pathogens leads to disinfestation. *P. ramorum* has multiple mechanisms that allow it to spread from host to host. Infection by *P. ramorum* does not always cause visual symptoms which could lead to difficulties in detection and persistence of infections (Parke and Lewis 2007). In soil, infestation can be particularly problematic because *P. ramorum* propagules can persist for as long as 33 months (Vercauteren et al. 2013). Within the soil profile, *P. ramorum* is most likely to be isolated from the organic layer within the top 5 cm of the soil profile (Dart et al. 2007). Soil disinfestation can be accomplished through fumigation; however, often nursery beds are not conducive to the effective use of fumigants due to the presence of gravel and/or compacted soil (Yakabe and MacDonald 2010). In recent years there has been a push towards non-chemical modes of soil disinfestation.

Non-chemical methods used to disinfest soil include treatments that target pathogens' sensitivity to heat. Soil solarization has been recommended as a management tool for the eradication of certain soil borne plant pathogens (Katan and Gamliel 2009; Funahashi and Parke 2015). Soil solarization is a passive method of heating soil that involves placing a transparent plastic film over moist soil, sealing the edges and allowing the sun to heat the soil beneath. The heat from the sun is trapped by the tarp and the soil is heated for an extended period of time in order to achieve temperatures that are lethal to target pathogens. The temperatures achieved during soil solarization are influenced by factors such as climate, physical soil properties, and the depth within the soil column.

Soil solarization could be useful against quarantined plant pathogens such as *P. ramorum* as well as other *Phytophthora* species. *P. pini* is a plant pathogenic species found in nurseries (Olson et al. 2012; Parke et al. 2014). *P. gonapodyides* and *P. chlamydospora* are species commonly found in water or wet soil that are generally considered saprophytic (Hansen et al. 2015) although they occasionally have been reported to cause disease (Corcobado et al. 2013; Hansen et al. 2015). Due to the quarantine status of *P. ramorum*, experimental soil solarization trials are limited to designated research facilities. Testing the viability of soil solarization as an eradication method for *Phytophthora* spp. in Oregon necessitates the use of surrogate species, such as those listed above.

The objectives of this research project were to 1) investigate and compare the thermal sensitivity of *Phytophthora* spp. and 2) to compare the efficacy of soil solarization at eliminating recovery of artificially introduced inoculum of *Phytophthora* between three species and between field sites in Corvallis, Oregon and San Rafael, California.

## **Materials and methods**

***Phytophthora* spp. inocula.** *Phytophthora* species used in this study included *P. ramorum* Werres, de Cock & Man in't Veld isolate Pr-1418886, *P. pini* Leonian (formerly *P. citricola* Sawada) isolate Pc98-517, *P. chlamydospora* Brasier & Hansen, and *P. gonapodyides* (Petersen) Buisman. All species were included in laboratory temperature gradient trials. Field trials at the Oregon State University Botany and Plant Pathology (OSU BPP) Farm included *P. pini* and *P. chlamydospora*; trials at the National Ornamental Research Site at the Dominican University of California (NORS-DUC) included *P. ramorum*, *P. pini*, and *P. chlamydospora*.

*Rhododendron grandiflorum* leaves were inoculated with selected *Phytophthora* species via the plug method. Leaves were rinsed with de-ionized water, dried, and wounded with a metal

puncturing tool (Fig. 1). Dilute (1/3 strength) V8 broth agar plugs colonized by each species were then placed atop wounds. These inoculated leaves were incubated in moist chambers at room temperature (19-21°) for two to three weeks as lesions developed. Leaf disc inoculum was prepared by excising discs from lesioned areas with a sterilized hole-punch (6mm diameter hole punch for field trials and 4mm diameter hole punch for temperature gradient trials).

Filter paper (Whatman No. 1, 11 µm opening) was cut into 4-mm diameter discs, autoclaved twice and placed onto the surface of media colonized by each species. The media used was pimarinic-ampicillin-rifamycin (PARP) (Jeffers and Martin 1986) supplemented with Terraclor (75% pentachloronitrobenzene; 66.7 mg liter<sup>-1</sup>) and hymexazol (25 mg liter<sup>-1</sup>) (PARPH). Cultures were incubated at room temperature for three to four weeks to allow colonization of the filter paper discs.

**Temperature gradient trials.** Leaf disc inocula and filter paper inocula were both used as substrates for temperature gradient trials. Ten inoculated disks of an individual species were placed in 200-µL Eppendorf tubes that were then filled to capacity with 245 µL de-ionized water. Four tubes of each species, for a total of sixteen tubes, were randomized and placed in each of the six temperature zones (30, 32, 34, 36, 38 or 40°C) within a thermocycler (Veriti™, Applied Biosystems, Foster City, California, USA) for 72 hours. Following heat treatment, discs were plated onto PARP selective media. Positive control discs were incubated in filled Eppendorf tubes at room temperature (19-21°C). Plated leaf discs were monitored for growth of *Phytophthora* over the following two weeks. The experiment was conducted twice for each inoculum substrate (filter paper or rhododendron leaf.)

**Solarization field experiments.** Solarization trials were performed at the Oregon State University Botany and Plant Pathology (OSU BPP) farm located east of Corvallis, Oregon and at



the National Ornamental Research Site at the Dominican University of California (NORS-DUC) in San Rafael, California. The NORS-DUC site is a facility for research on quarantined plant pathogens including *Phytophthora ramorum*.

Each of the field sites had eight 6.25 m<sup>2</sup> square plots of exposed soil, which were irrigated to saturation and allowed to drain overnight. Leaf discs (6-mm diameter) were excised from *Rhododendron grandiflorum* leaves inoculated with each species. Infested leaf discs were placed in mesh sachets, 10 discs per sachet, and placed within soil filled columns at 0 cm, 5 cm, and 15 cm depths (Fig. 2.b). Columns (8 cm diameter x 24 cm depth) and sachets (4 cm x 4 cm) were constructed from nylon phytoplankton netting (Aquatic Eco-systems, Apopka, Florida) with a mesh opening of 105 µm. Once filled with soil, two columns for each species were placed into the ground in each of the eight plots (Fig. 2.c). Columns were placed in cylindrical holes (12 cm diameter x 30 cm deep) and arranged radially around the center point of each plot such that each column was 45 cm from the center point (Fig. 2.a). Four randomly selected plots were covered with a clear 6-mil-thick polyethylene film treated with an anti-condensation coating (Thermax™, AT Films, Edmonton, Alberta, Canada ), held in place by a 6"-wide border of 3/4 inch crushed rock. The four remaining plots were left uncovered to serve as non-solarized controls. Field trials began at the Oregon field site on July 24<sup>th</sup>, 2014 and at the California field site on July 30<sup>th</sup>, 2014.

One column per species per plot was retrieved following two and four weeks of treatment. Retrieval from solarized plots following two weeks of treatment was conducted by cutting open the clear plastic tarp to remove columns and resealing cuts with clear plastic tape in order to continue solarizing the designated four week samples. Following retrieval, leaf discs were rinsed with de-ionized water and plated on PARPH selective media. Retrieved leaf discs

plated on the selective medium were observed over a two to three week period for signs of pathogen growth. Leaf inoculum from storage was plated on PARPH at the same time as the retrieved samples for morphological comparison. Samples were recorded as a positive recovery when they had identical colony morphologies and spores to known pathogen samples.

**Temperature data collection.** “iButton” data loggers (iButton, Thermocron, Baulkham Hills, New South Wales, Australia) were programmed to collect temperature data every 30 minutes over the total four week treatment period. Data loggers were placed within protective hard plastic containers (Aqua Lab, Decagon, Pullman, Washington, USA) which were then placed within clear plastic zip lock bags; iButtons placed at 0 cm were also wrapped in aluminum foil to avoid additional heating via the greenhouse effect. Data loggers were placed within randomly selected columns in each plot within the four week treatment group, such that each plot had one data logger at each of the three depths. Compiled data were processed in Excel to obtain basic temperature statistics such as averages, maxima, minima, amplitude and cumulative hours over 35°C (the estimated threshold temperature) and to chart temperature fluctuations over time.

**Statistical analysis.** Statistical analysis of results from temperature gradient and solarization field trials were carried out using R statistical software (version 3.1.1).

Temperature gradient data was fit to a probit model describing the relationship between temperature and binary recovery response for each individual species using the `glm( )` function. Family was set to quasibinomial to account for overdispersion in our dataset. This model was then used to calculate the temperature dose required to eliminate 99.9% of recovery among individual *Phytophthora* spp. (LD<sub>99.9</sub>). Data were combined for all trials when no significant trial and temperature interaction was detected at  $\alpha = 0.05$ .

A probit model was also used to analyze differences between species response in the solarization experiments. Analyses were run for all non-zero treatment groups in order to detect differences in recovery between species, given their location, treatment, depth, and duration of treatment.

## Results

### *Temperature gradient trials*

**LD<sub>99.9</sub> estimates for *Phytophthora* spp.** Trials using filter paper inocula were combined for *P. gonapodyides* and *P. pini*, while trials using rhododendron leaf inocula were combined for *P. chlamydospora* and *P. ramorum*.

The temperature treatment predicted to be lethal for 99.9% of samples (LD<sub>99.9</sub>) varied between individual species and between the substrate used (Table 1 and Fig. 6). Results from temperature gradient trials using inoculated filter paper indicated that *P. gonapodyides* is most resilient and *P. ramorum* is least resilient following exposure to high temperatures, while results from leaf inoculum trials indicated that *P. chlamydospora* is most resilient and *P. pini* is least resilient following exposure to high temperatures.

Estimates generated from trials using filter paper inocula indicated the LD<sub>99.9</sub> *P. gonapodyides* (Fig. 4), *P. pini* (Fig. 5), *P. chlamydospora* (Fig. 3), and *P. ramorum* (Fig. 6) are 41.55°C, 41.23°C, between 38.27 and 40.31°C, and between 34.46 and 38.39°C respectively. LD<sub>99.9</sub> estimates from trials using leaf disc inocula for *P. chlamydospora*, *P. gonapodyides*, *P. ramorum*, and *P. pini* were 47.66°C; between 42.47 and 45.69°C; 41.55 °C; and between 37.98 and 39.67°C respectively.

For most species, the estimated LD<sub>99.9</sub> were greater in trials using rhododendron leaf inocula than in trials using filter paper inocula. *P. pini* was the only species that had a higher

estimated LD<sub>99,9</sub> in trials using filter paper inocula. The differences between the LD<sub>99,9</sub> estimates for the two types of inocula used were between 7.4-9.4°C for *P. chlamydospora*, 3.2-7.1°C for *P. ramorum*, 1.6-3.3°C for *P. pini*, and 0.1-3.1°C for *P. gonapodyides*.

The rate of recovery for positive controls was 100% with the exception of the *P. ramorum* positive control for the second filter paper trial in from which the pathogen was recovered from only 7 of the 10 discs.

### ***Solarization field trials***

**Recovery of *Phytophthora* spp. following solarization.** *Phytophthora* spp. were not recovered from leaf disc inocula buried in solarized plots at 0, 5, or 15 cm depths in Oregon or buried at 0 or 5 cm depths in California (Fig. 7). The only *Phytophthora* spp. to be recovered from buried inocula in solarized plots were from inocula of *P. chlamydospora* buried at 15 cm in California, which were recovered at both time points. At the surface, the only *Phytophthora* spp. to be recovered was *P. pini*, which was recovered after two weeks in both Oregon and California, but only in nonsolarized plots. Statistical analysis was only needed to compare non-zero treatment groups, which were primarily groups from nonsolarized plots. Among nonsolarized plots, the only significant differences in recovery between species were found in the group of samples at the Oregon field site which were buried at 5 cm for two weeks (Table 2, P-value: 0.0045, probit) and for the group of samples at the California field site which were buried at 15 cm for two weeks (Table 2, P-value < 0.0001, probit).

**Soil temperatures from field experiments.** Temperature data were divided into three subsets by depth (Table 3). At the surface, the average temperature in solarized plots was 14.8-15.6 °C greater than in nonsolarized plots and samples were exposed to an additional 128-149 cumulative hours over 35 °C. At 5 cm depth, the average temperature was increased by 9.4-11.4

°C in solarized plots and the cumulative number of hours over 35 °C were increased by 255-286 hours. At 15 cm depth, the average temperature was increased by 6.8-6.9 °C in solarized plots and the cumulative number of hours over 35 °C was increased by 271-300 hours. The amplitude of diurnal temperature fluctuations decreased with depth, with greater maximal and lower minimal temperatures recorded at 0 cm and 5 cm than at those recorded at 15 cm. Although the difference in the average temperature diminished with increasing depth, the difference between solarized and nonsolarized plots in the number of hours over 35 °C was greater with increasing depth.

## Discussion

The estimated lethal dose ( $LD_{99,9}$ ) for each species was different depending on the species and the substrate type used in the temperature gradient trial. The differences between species were expected considering their described optimal growth temperatures. *P. chlamydospora* has been described as having a growth optimum of 25-28°C with a maximum temperature for growth between 36 and 37°C (Hansen, Sutton, and Reeser 2015). The maximal growth temperature of both *P. gonapodyides* and *P. pini* has been reported as 35°C, with *P. gonapodyides* displaying slow growth between 20-30°C and *P. pini* having an optimal growth temperature of 25°C (Hong et al. 2011; Brasier et al. 1993). *P. ramorum* is described as being able to grow at temperatures between 2-30°C, with 20°C being the optimal temperature for growth (Werres et al. 2001).

For most species,  $LD_{99,9}$  estimates in trials using rhododendron leaves were higher than in those using filter paper, with the exception of *P. pini*. Differences between estimates were greater in general for *P. ramorum* (+7.4-9.4°C for leaf inoculum) and *P. chlamydospora* (+3.2-7.1°C for leaf inoculum) than they were for *P. pini* (-1.6-3.3°C for leaf inoculum) and *P. gonapodyides* (+0.1-3.1°C for leaf inoculum). The greater recovery and resulting higher  $LD_{99,9}$  estimates from trials using rhododendron leaves observed with *P. ramorum* and *P. chlamydospora* could be attributed to production of chlamydospores, asexual survival spores which both species are known to produce (Hansen et al. 2015; Werres et al. 2001). *P. ramorum* is especially well known to produce abundant chlamydospores, both in high nutrient media and in plant tissue (Tooley et al. 2004; Lewis et al. 2004; Werres et al. 2001). *P. gonapodyides* and *P. pini* do not produce chlamydospores (Brasier et al. 1993; Hong et al. 2011), which could explain the smaller differences in  $LD_{99,9}$  estimates between the two trial types in these species. *P. pini* is

known to produce oospores, thick-walled sexual spores considered survival structures, however *P. gonapodyides* is not.

It seems likely that using rhododendron leaves would lead to a greater abundance of chlamydospores among chlamydospore-producing *Phytophthora* spp. because rhododendron leaves would represent an environment with greater nutrient availability than filter paper. The original motivation behind using filter paper instead of rhododendron leaf inoculum was to have a more standardized medium for pathogens to grow into, avoiding the structural variation that could be found between individual leaves and between leaves in different seasons. Trials using leaf inoculum are potentially more representative of real life conditions. Leaves provide more nutrients for *Phytophthora* species than filter paper; however, using filter paper standardizes the inoculum and facilitates the quantification of survival structures, such as chlamydospores and oospores. While chlamydospores and oospores are known as survival structures, it remains unclear why in some cases species that do not produce these structures such as *P. gonapodyides*, are more resistant to heat than those that produce them abundantly, such as *P. ramorum* (Werres et al. 2001; Brasier et al. 1993). A potential avenue of future research would be to investigate which structures are most important for the survival of *Phytophthora* spp. following thermal stress and whether an increased abundance of chlamydospores or oospores could be related to a higher rate of recoverability.

While temperature gradient trials were useful in making comparisons between the heat sensitivities of different species, they are not exactly representative of temperature conditions which would occur in the field. In field trials, temperature fluctuations were diurnal, with the high temperatures of the day alternating with cooler temperatures at night. It's possible that these nightly lows allow pathogens to avoid the rapid accumulation of damage which they experience

during the high temperatures of the day. Intermittent heat exposure is less damaging than exposures to constant heat (Funahashi 2015), duration and nature (constant vs. intermittent) of heat exposure should be taken into account when considering the estimated LD<sub>99.9</sub> temperatures presented.

Lethal dose estimates and their associated standard errors (Table 1) were generated based on a line fit to the curve of pathogen recovery data. Standard error values in this case are not mean errors, but rather indicative of the predictive power of the model. In some cases, the curve generated was very steep and the error associated with the LD<sub>99.9</sub> estimate was very large. This was the case for *P. chlamydospora* in trial 1 using filter paper inocula, with a standard error estimate of  $\pm 14.39^{\circ}\text{C}$ . This large error was the consequence of the model predicting that a small change in temperature would result in a big difference in the estimated lethality of the dose.

Estimated LD<sub>99.9</sub> temperatures from trials using rhododendron leaves indicate that *P. chlamydospora* can survive following exposure to very high temperatures, which is consistent with results from my solarization trials. In field trials, *P. chlamydospora* was the only species to be recovered from solarized plots as well as the most recovered species in trials in which the differences between species were statistically significant (Table 2 and Fig. 8). The pathogen recovery results from this trial correspond somewhat with 2012 field trials conducted at the same field sites with solarization films of the same material and dimensions (Funahashi and Parke 2015). In 2012 trials, *P. pini* was used in Oregon trials and *P. ramorum* was used in California trials, so it was challenging to make conclusions about the differences between species in response to treatment with solarization. The results from my trial showed similar trends to this earlier trial in that *P. ramorum* was eliminated from the top 15 cm in solarized plots in the California trial and *P. pini* was eliminated from the top 5 cm in Oregon trials, however in my



solarization trial *P. pini* was also eliminated from 15 cm in solarized plots in Oregon, a depth from which it was recovered in solarized plots in Oregon the previous study. This difference could be attributed to the timing of the solarization trials, because while my Oregon trial began in late July and continued through late August, the previous study began in late August and continued through late September. Temperatures achieved by soil solarization are directly related to the intensity of solar radiation, therefore this difference in timing could have been a factor in the survival of *P. pini* from 15 cm in the previous trial.

From previous studies, we know that *P. pini* and *P. chlamydospora* generally have higher heat tolerances than *P. ramorum* (Hong et al. 2011; Hansen et al. 2015; Werres et al. 2001), which is consistent with results from my solarization trials, although these differences were not always statistically significant. *P. pini* and *P. chlamydospora* both have potential for use as indicator species for the elimination of *P. ramorum* in soil solarization studies outside of quarantine sites. With generally higher thermotolerances, successfully eradicating artificially introduced inoculum of *P. pini* and *P. chlamydospora* indicates that if *P. ramorum* were present it could most likely be eradicated by a similar treatment.

This study indicates that soil solarization is a promising treatment for nursery beds infested with *Phytophthora* spp. in both Oregon and California, especially if conducted during the hottest months of the year. More research is needed to investigate the specific response of different *Phytophthora* species to heat treatments such as soil solarization; to build a predictive model to estimate the temperatures that could be achieved through soil solarization in different climates and in different types of soils; to better understand the thermal tolerances of different *Phytophthora* species; as well as to understand roles played by different survival structures.

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## Tables and Figures

Table 1. LD<sub>99,9</sub> estimates ( $\pm$  standard error) for individual temperature gradient trials

Substrate	Trial	Species	LD <sub>99,9</sub> ( $\pm$ s.e.)	Difference from first trial
Filter paper	Trial 1	<i>P. chlamydospora</i>	38.27 ( $\pm$ 14.39)	
		<i>P. gonapodyides</i>	41.66 ( $\pm$ 2.26)	
		<i>P. pini</i>	37.09 ( $\pm$ 1.02)	
		<i>P. ramorum</i>	38.39 ( $\pm$ 1.66)	
	Trial 2	<i>P. chlamydospora</i>	40.31 ( $\pm$ 1.34)	+2.04
		<i>P. gonapodyides</i>	43.12 ( $\pm$ 1.71)	+1.46
		<i>P. pini</i>	41.43 ( $\pm$ 0.90)	+4.34
		<i>P. ramorum</i>	34.46 ( $\pm$ 0.29)	-3.93
	Combined Trials	<i>P. pini</i>	41.23 ( $\pm$ 1.01)	
		<i>P. gonapodyides</i>	42.55 ( $\pm$ 1.25)	
Rhododendron leaf	Trial 3	<i>P. chlamydospora</i>	46.07 ( $\pm$ 2.10)	
		<i>P. gonapodyides</i>	45.69 ( $\pm$ 2.75)	
		<i>P. pini</i>	39.67 ( $\pm$ 1.47)	
		<i>P. ramorum</i>	38.36 ( $\pm$ 1.06)	
	Trial 4	<i>P. chlamydospora</i>	47.41 ( $\pm$ 3.00)	+1.33
		<i>P. gonapodyides</i>	42.47 ( $\pm$ 1.43)	-3.22
		<i>P. pini</i>	37.98 ( $\pm$ 0.39)	-1.69
		<i>P. ramorum</i>	42.34 ( $\pm$ 2.10)	+3.98
	Combined Trials	<i>P. ramorum</i>	41.55 ( $\pm$ 1.51)	
		<i>P. chlamydospora</i>	47.66 ( $\pm$ 2.08)	

Table 2. Probit results from comparison between species, for given location, treatment, duration, and depth.

State	Treatment	Duration	Depth (cm)	P-value
Oregon	nonsolarized	2 weeks	5	0.0045
Oregon	nonsolarized	4 weeks	5	0.2446
Oregon	nonsolarized	2 weeks	15	0.9643
Oregon	nonsolarized	4 weeks	15	0.2203
California	nonsolarized	2 weeks	5	0.1334
California	nonsolarized	4 weeks	5	0.7909
California	nonsolarized	2 weeks	15	0.0000
California	nonsolarized	4 weeks	15	0.5450

Table 3. Summary of temperature data from iButton temperature sensors

Location Treatment Duration Depth	Corvallis, Oregon				San Rafael, California			
	Nonsolarized		Solarized		Nonsolarized		Solarized	
	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
0 cm								
Average Temperature ( °C)	26.84	26.82	43.82	42.45	24.35	24.90	39.05	39.66
Standard Error ( $\pm$ °C)	0.23	0.16	0.43	0.31	0.18	0.13	0.36	0.25
Maximum Temperature ( °C)	48.50	50.00	79.00	79.00	48.00	49.00	80.00	81.50
Minimum Temperature ( °C)	10.00	10.00	14.00	14.00	11.50	10.00	18.00	18.00
Amplitude (Max - Min) (°C)	38.50	40.00	65.00	65.00	36.50	39.00	62.00	63.50
Average hours > 35°C	106.33	178.17	189.83	327.00	73.25	169.00	141.00	297.13
5 cm								
Average Temperature ( °C)	27.81	27.95	40.16	39.33	25.86	26.57	35.37	35.97
Standard Error ( $\pm$ °C)	0.12	0.09	0.30	0.21	0.10	0.07	0.15	0.10
Maximum Temperature ( °C)	41.50	56.00	77.00	77.00	35.50	37.00	52.50	52.50
Minimum Temperature ( °C)	14.00	14.00	14.00	14.00	18.50	18.50	21.00	21.00
Amplitude (Max - Min) (°C)	27.50	42.00	63.00	63.00	17.00	18.50	31.50	31.50
Average hours > 35°C	54.83	93.00	202.83	347.50	2.50	39.17	147.25	325.25
15 cm								
Average Temperature ( °C)	27.40	27.76	34.72	34.63	26.26	26.92	33.07	33.75
Standard Error ( $\pm$ °C)	0.07	0.06	0.09	0.06	0.04	0.03	0.07	0.04
Maximum Temperature ( °C)	32.50	54.50	42.00	42.00	31.50	32.00	40.50	41.00
Minimum Temperature ( °C)	18.00	16.50	20.50	20.50	22.00	22.00	23.50	23.50
Amplitude (Max - Min) (°C)	14.50	38.00	21.50	21.50	9.50	10.00	17.00	17.50
Average hours > 35°C	0.00	8.67	184.67	308.50	0.00	0.00	117.50	270.88



Figure 1. Wounded leaf (left) and leaf wounding tool (right).

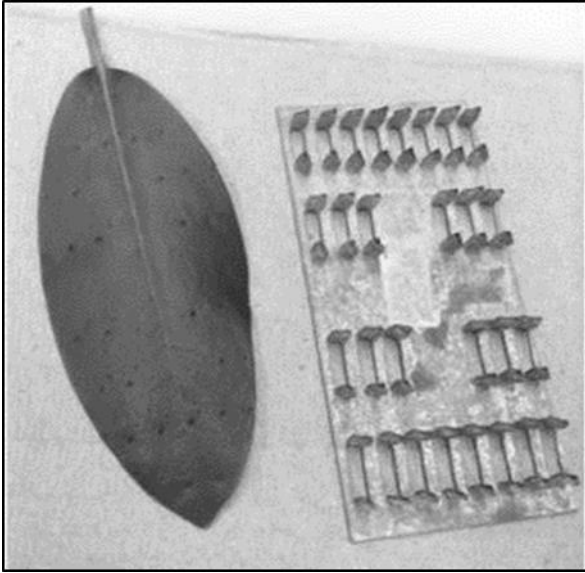


Figure 2. Cylindrical holes arranged radially around the center point of each plot (a), empty columns and sachets (b), and filled column with inoculum-filled sachet on surface (c).

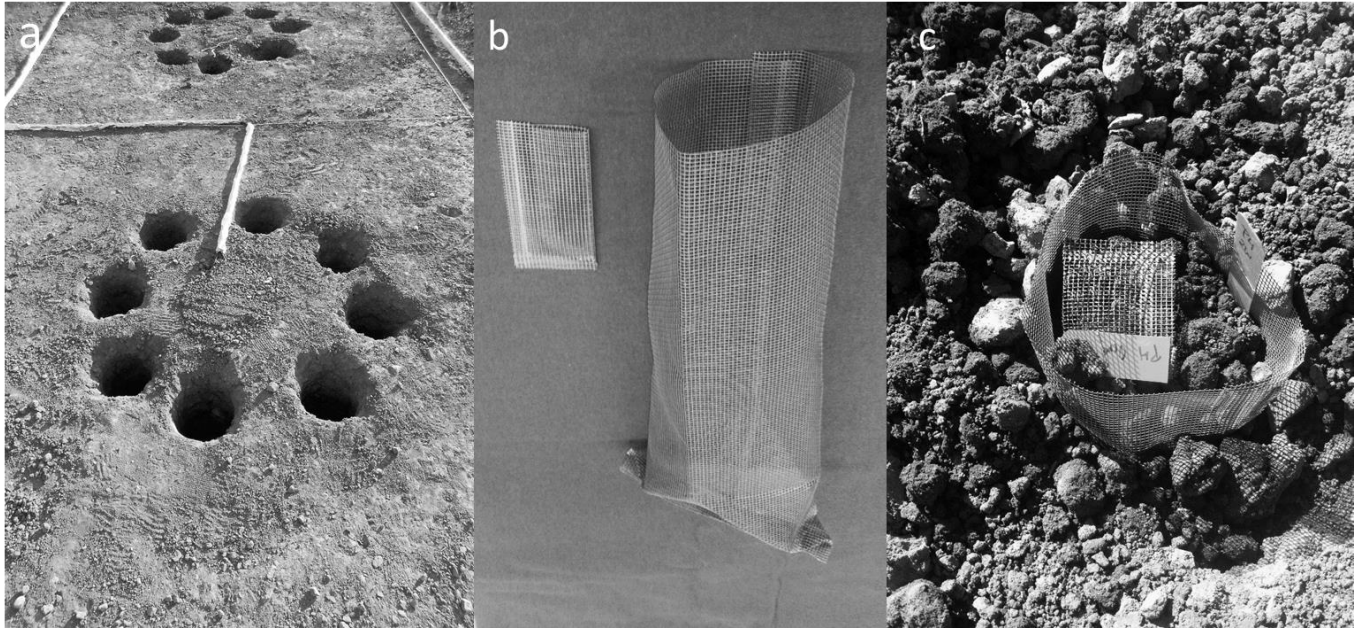


Figure 3. Plotted line of LD<sub>99.9</sub> estimates for *P. chlamydospora* from temperature gradient trials with filter paper (top) and with rhododendron leaves (bottom).

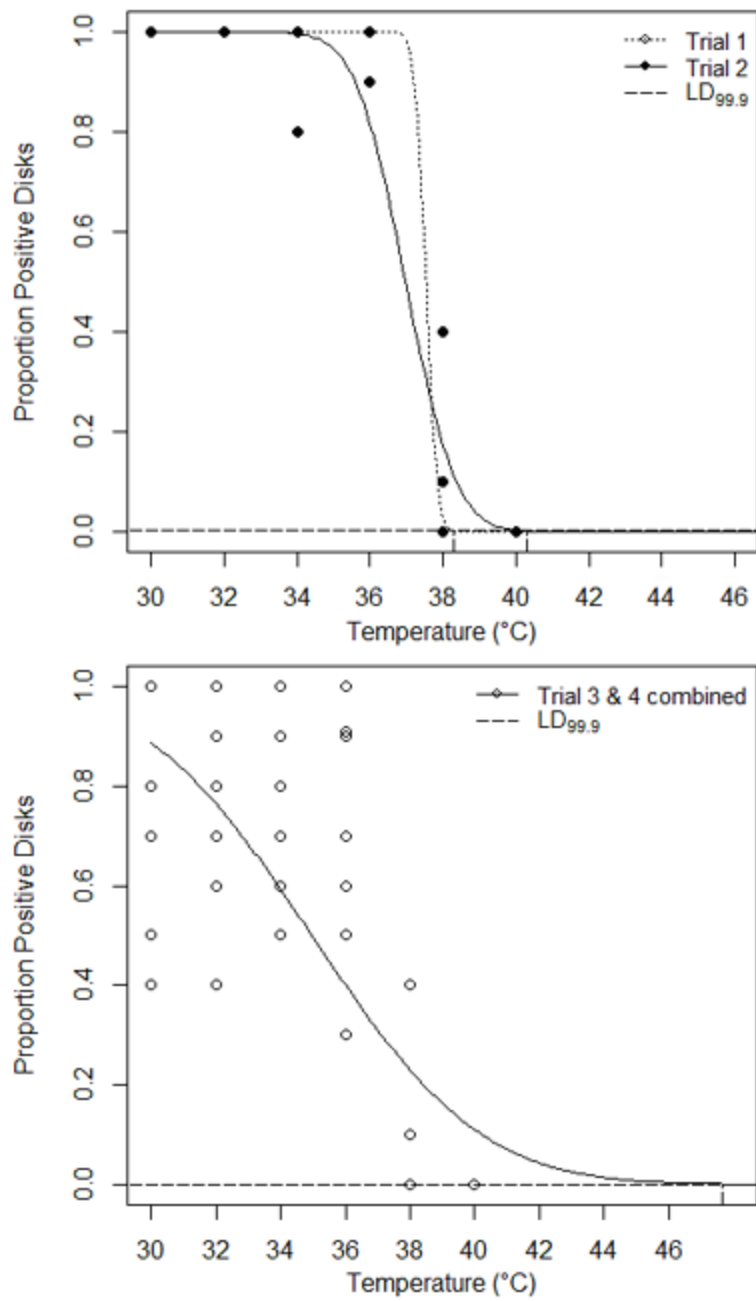


Figure 4. Plotted line of LD<sub>99.9</sub> estimate for *P. gonapodyides* from temperature gradient trials with filter paper (top) and rhododendron leaves (bottom).

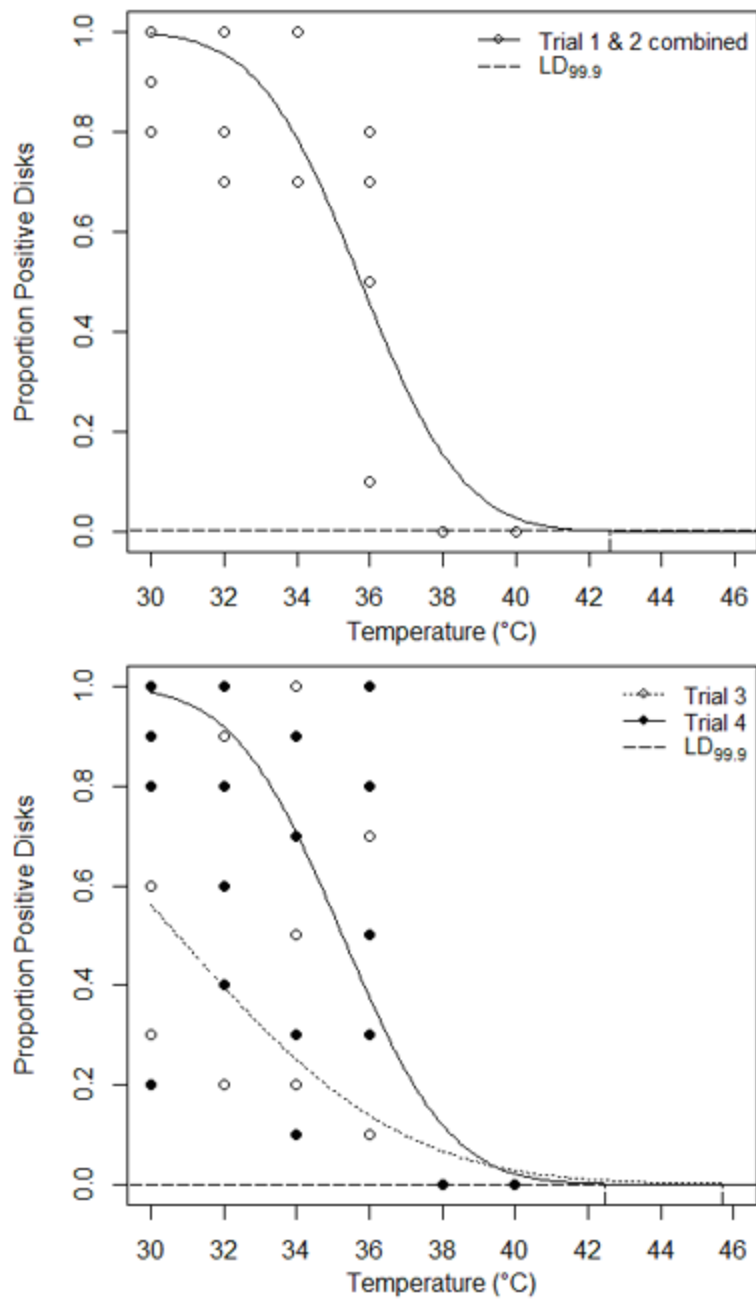


Figure 5. Plotted line of LD<sub>99.9</sub> estimate for *P. pini* from temperature gradient trials with filter paper (top) and rhododendron leaves (bottom).

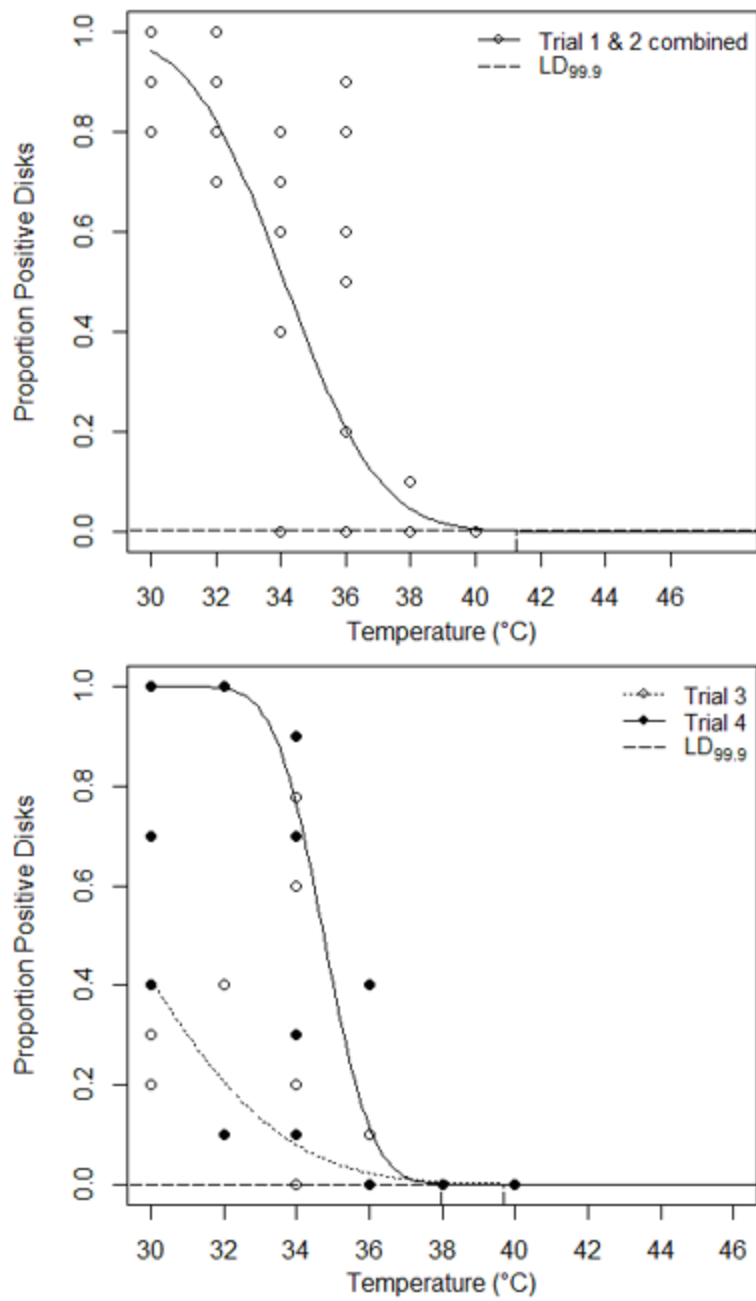


Figure 6. Plotted line of LD<sub>99.9</sub> estimate for *P. ramorum* from temperature gradient trials with filter paper (top) and rhododendron leaves (bottom).

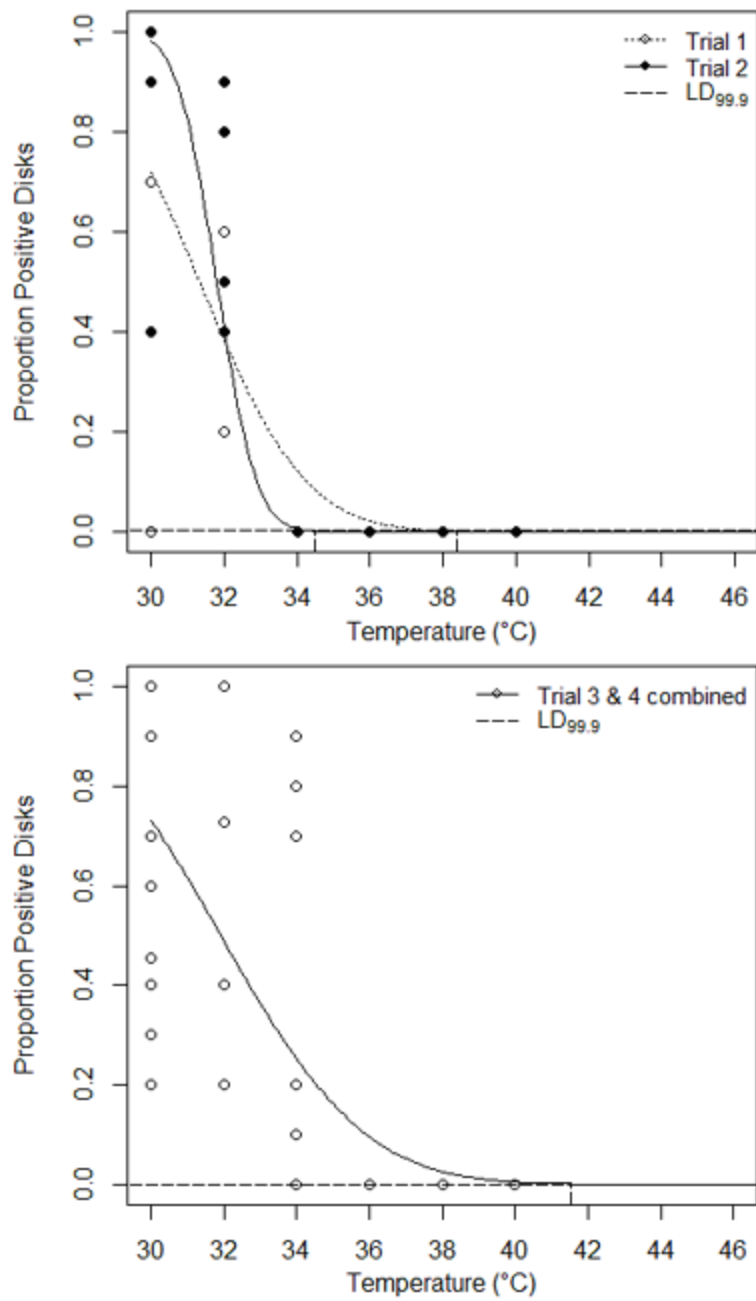


Figure 7. Comparison of calculated LD<sub>99.9</sub> estimates by species

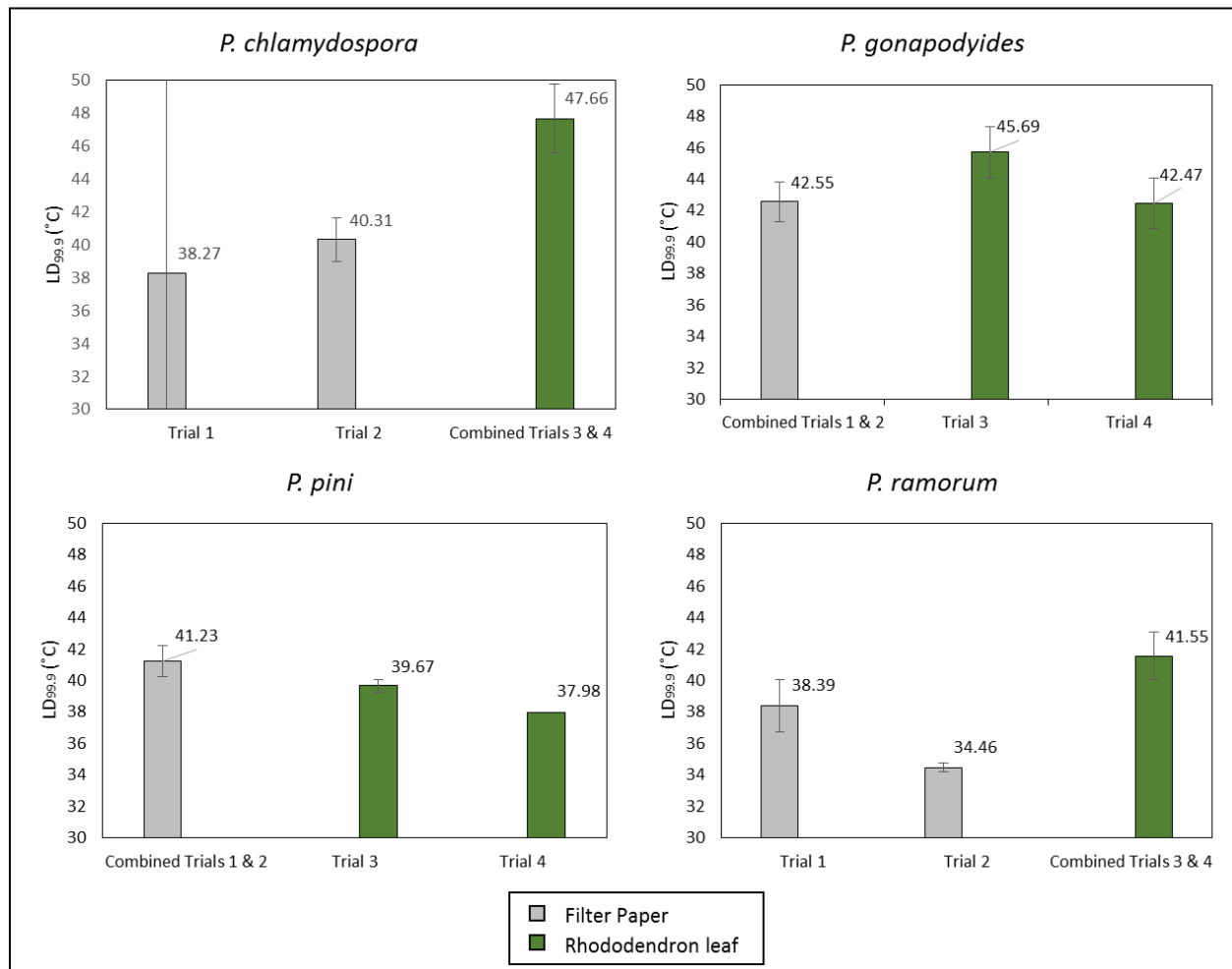
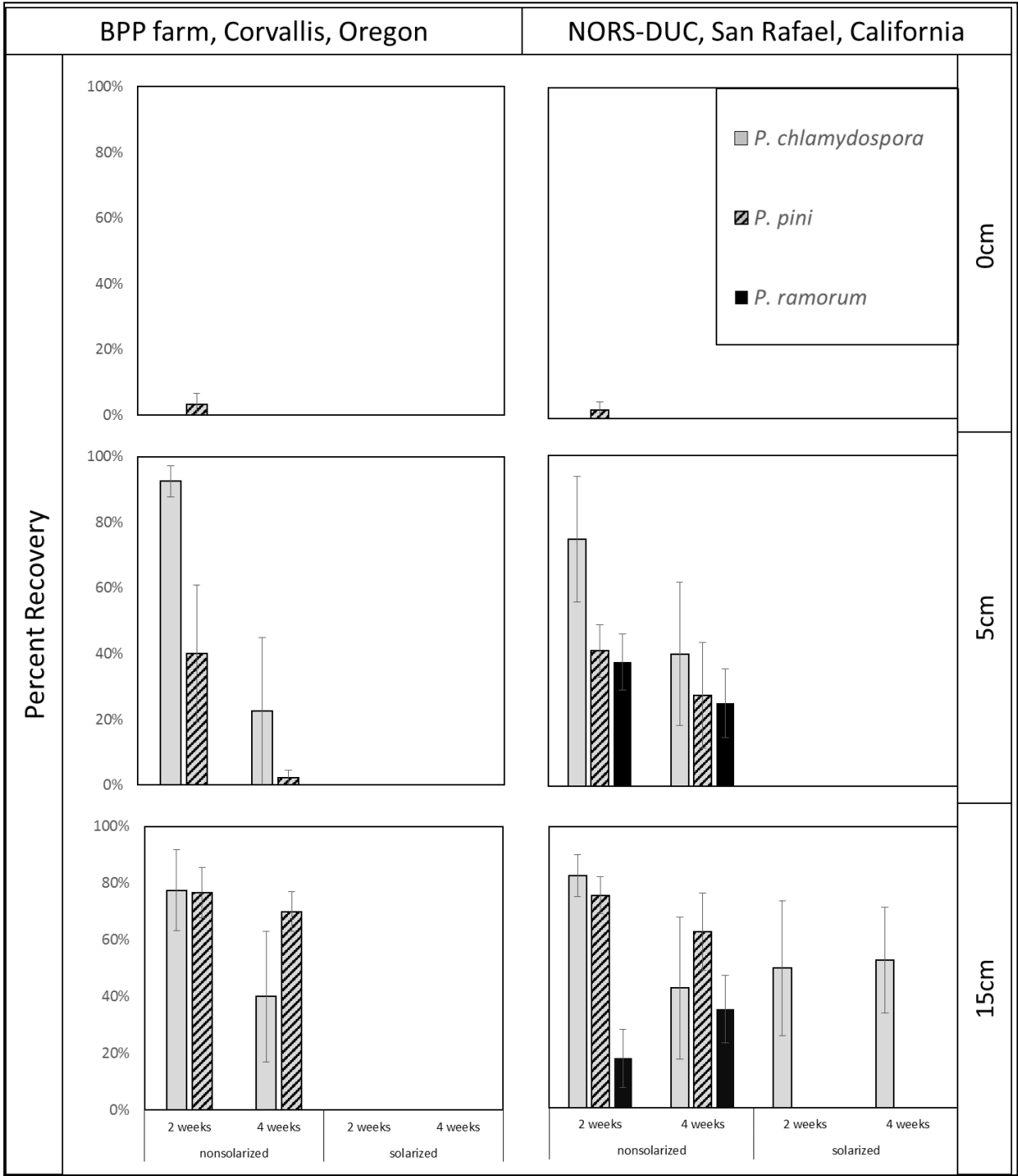


Figure 8. Summary of recovery data from solarization field trials conducted in Oregon and California





## Appendix I: Duration response

### Objective:

Compare *Phytophthora* spp. to each other in terms of their response to thermal stress of different durations.

### Procedure:

- Prepare leaf inoculum of different *Phytophthora* species via agar plug method. Once inoculum has been incubated at room temperature (19-21°C) for 2-3 weeks, excise leaf discs (4 mm diameter) using a small hole punch or cork borer.
- Fill 25 tubes (200 µL Eppendorf tubes) with ten leaf discs for each species (if using four species, the total number of tubes will be 100). To distinguish between different species use tubes of different colors or mark tubes with different colors.
- Randomize four tubes of each species (16 tubes total).
- Fill tubes to capacity with de-ionized water (approximately 245 µL).
- Place 16 randomized tubes in a Veriti thermocycler in the first duration zone (see figure I.4). Set the Veriti thermocycler to heat tubes to 36 °C for 12 hours.
- Store additional tubes at 4 °C. Every 12 hours repeat steps 3-5, adding 16 tubes at a time, until all but four tubes have been used.
- The additional four tubes (one tube per species) are positive controls.
- When the last set of 16 tubes has been heated for 12 hours (and the first set has been heated for a total of 72 hours) remove all tubes from the thermocycler. Place tubes in order onto a PCR tube rack which has an A-H/1-12 grid (such that the tube in the upper left hand corner of the rack is in position A1).

- Plate discs onto PAR selective media (1 tube/10 discs per plate). Record species and tube position (A1 – H12) for each plate.
- Record frequency of recovery over the following two weeks.

Note: For trial 1 the amount of de-ionized water added to tubes was 100  $\mu$ L; for trials 2 and 3 the amount was altered to 245  $\mu$ L.

### **Observations:**

Trial 1: *P. ramorum* was recovered in the highest proportion after 12 hours of heat exposure, with recovery greatly reduced or eliminated for durations of 36 hours or more. Other species were recovered in higher proportions after 24 hours than 12 hours, but recovery was reduced for exposures greater than 24 hours.

Trial 2: *P. ramorum* and *P. pini* showed similar recovery trends. *P. ramorum* and *P. pini* both showed the greatest recovery after 12 hours of exposure, and were recovered infrequently following 36 or more hours of heat exposure. *P. gonapodyides* and *P. chlamydospora* were recovered at a very high frequency following 12-36 hours of heat exposure. The recovery of *P. gonapodyides* between 48-72 hours of exposure gradually decreased. *P. chlamydospora* was recovered at a very low frequency between 48 and 60 hours of heat exposure, but at a higher frequency following 72 hours of exposure.

Trial 3: *P. chlamydospora* has high recovery exposed to heat for 12-60 hours, with a reduction in recovery for samples exposed to heat for 72 hours. *P. gonapodyides* has high recovery for samples exposed to heat for 12-48 hours, with a reduction in recovery after 72 hours. *P. pini* has

moderate recovery following 12-24 hours of exposure, but recovery is reduced for durations between 36 and 72 hours. *P. ramorum* has no recovery for durations between 12-24 hours, but is recovered in higher frequencies with longer durations between 12-72 hours of exposure.

**Figures:**

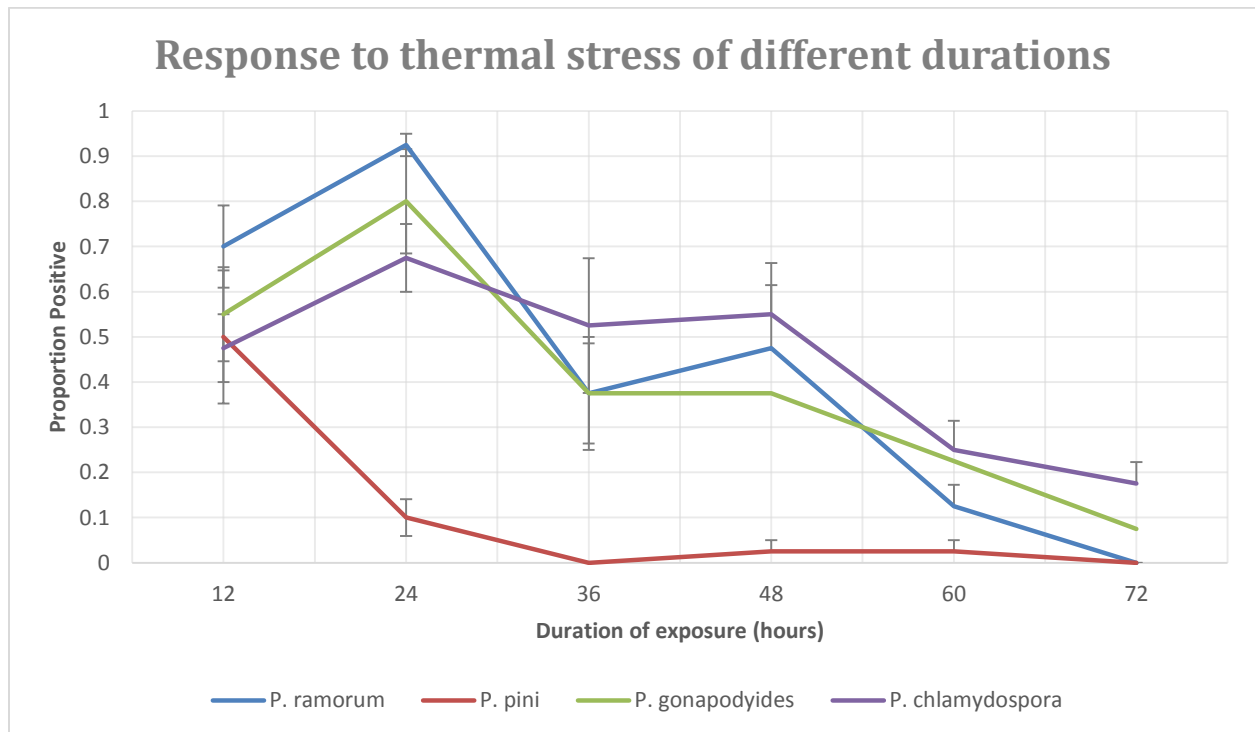


Figure I.1: trial 1 summary

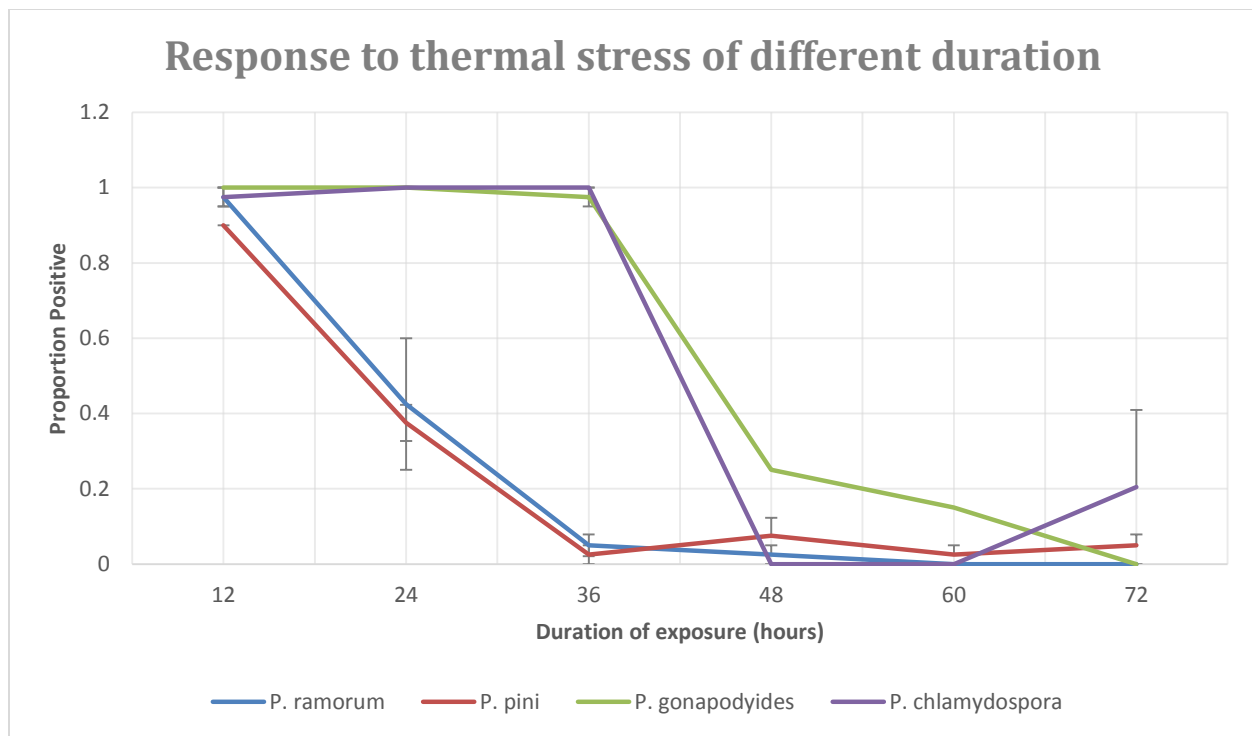


Figure I.2: trial 2 summary

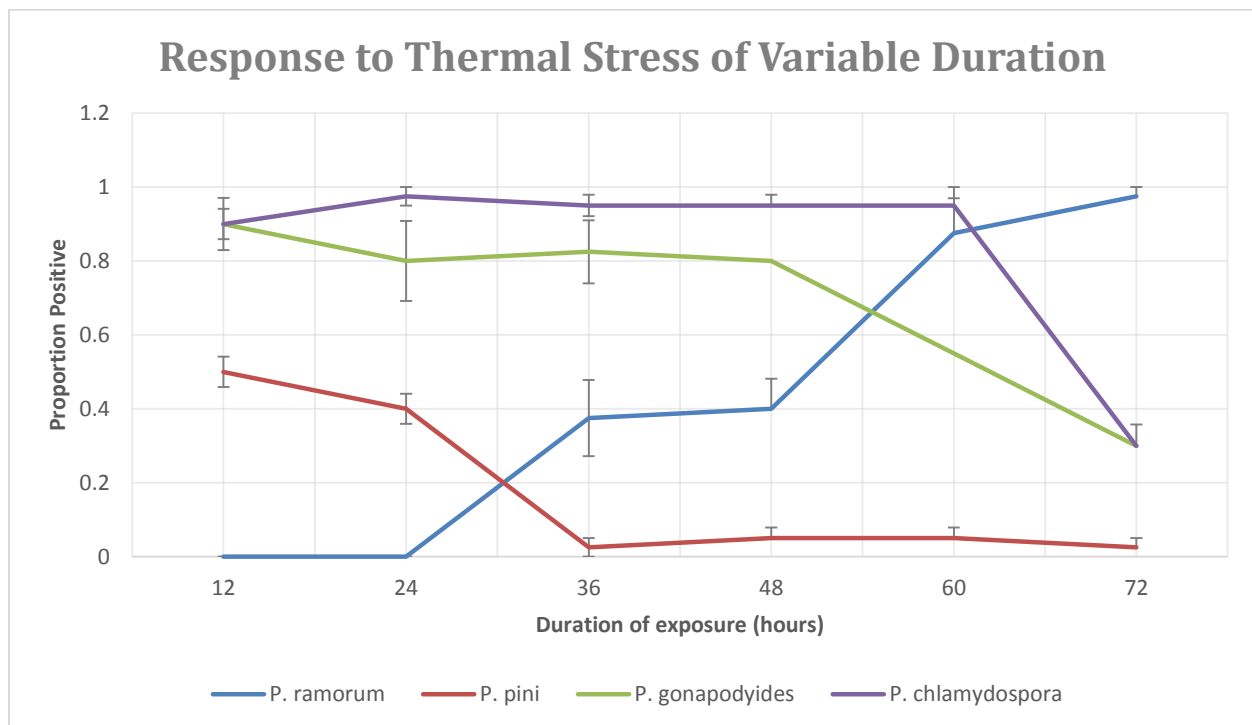


Figure I.3: trial 3 summary

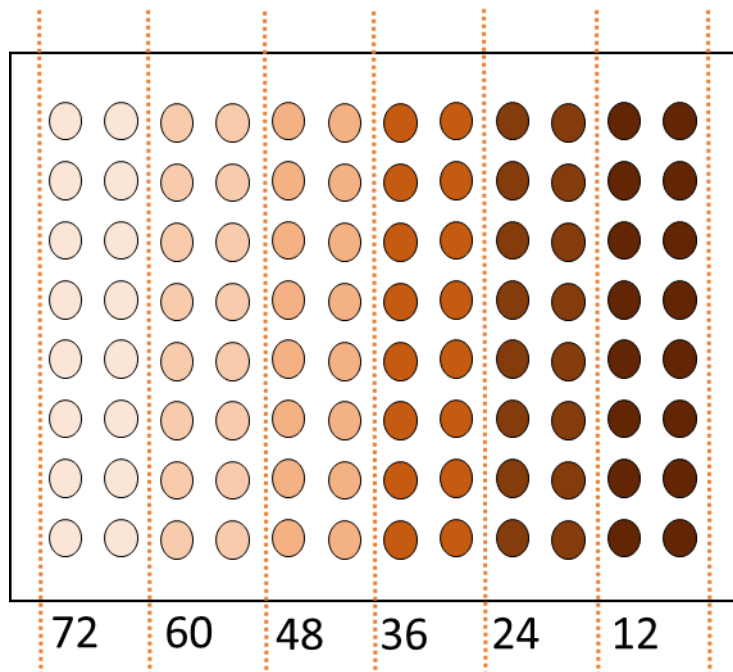


Figure I.4: Different duration zones ranging from 12-72 hours

## Appendix II: Diurnal flux response

### Objectives:

To simulate and investigate possible effects of daily temperature fluctuations on the recovery of *Phytophthora* species exposed to high temperatures for varying amounts of time.

### Procedure:

- Prepare leaf inoculum of different *Phytophthora* species via agar plug method. Once inoculum has been incubated at room temperature (19-21°C) for 2-3 weeks, excise leaf discs (4 mm diameter) using a hole punch or cork borer.
- Fill 25 tubes (200 µL Eppendorf tubes) with ten leaf discs for each species (if using four species, the total number of tubes will be 100). To distinguish between different species use tubes of different colors or mark tubes with different colors.
- Randomize four tubes of each species, 16 tubes total. Repeat process for two sets of 16 tubes.
- Keeping sets of 16 separate, fill tubes to capacity with de-ionized water (approximately 245 µL).
- Place 16 randomized tubes in Veriti thermocycler in the first zone (see figure I.4). Set the Veriti thermocycler to heat tubes to 36 °C for 12 hours and then cool to 20 °C for 12 hours.
- Place 16 randomized tubes in Veriti thermocycler in the second zone (see figure I.3). Set the Veriti thermocycler to heat tubes to 38 °C for 12 hours and then cool to 20°C for 12 hours.

- Store additional tubes at 4 °C. Every 24 hours repeat steps 3-5, adding 16 tubes at a time, until all but four tubes have been added to the machine.
- The additional four tubes (one tube per species) are positive controls.
- When the last two sets of 16 tubes have been through the heat cycle for 24 hours (and the first set has been heated for a total of 72 hours) remove all tubes from the thermocycler. Place tubes in order onto a PCR tube rack which has an A-H/1-12 grid (such that the tube in the upper left hand corner of the rack is in position A1).
- Plate discs onto PAR selective media (1 tube/10 discs per plate). Record species and tube position (A1 – H12) for each plate.
- Record frequency of recovery over the following two weeks.

### **Observations:**

The slopes of the curves for species fluctuating between 38/20 were steeper than those for the same species fluctuating between 36/20 on plots tracking proportion recovery (0-1) vs. duration of exposure (24-72 hours). The proportion recovery was lower for samples fluctuating between 38/20 °C than it was for samples fluctuating between 36/20 °C.

**Figures:**

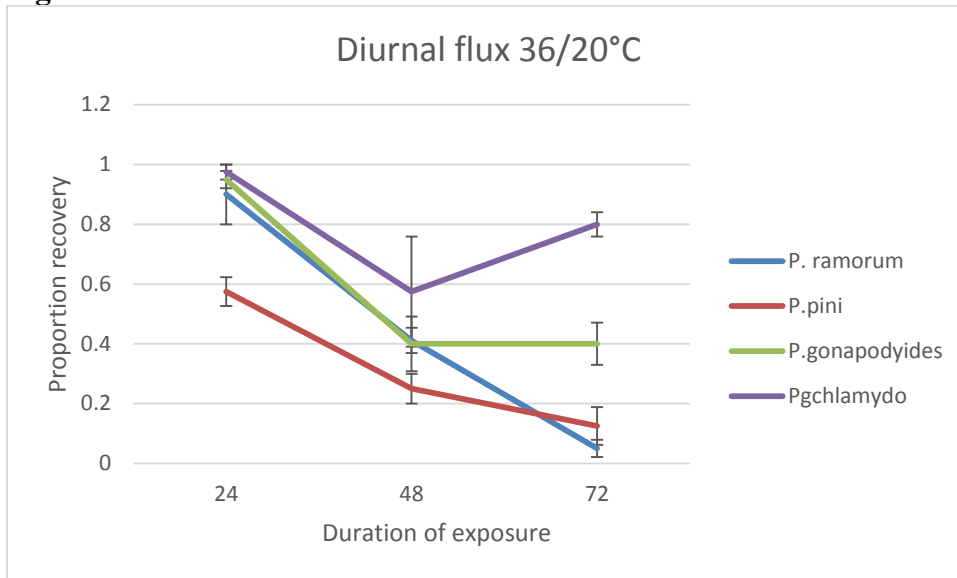


Figure II.1: Recovery of species from diurnal flux between 36 °C and 20 °C

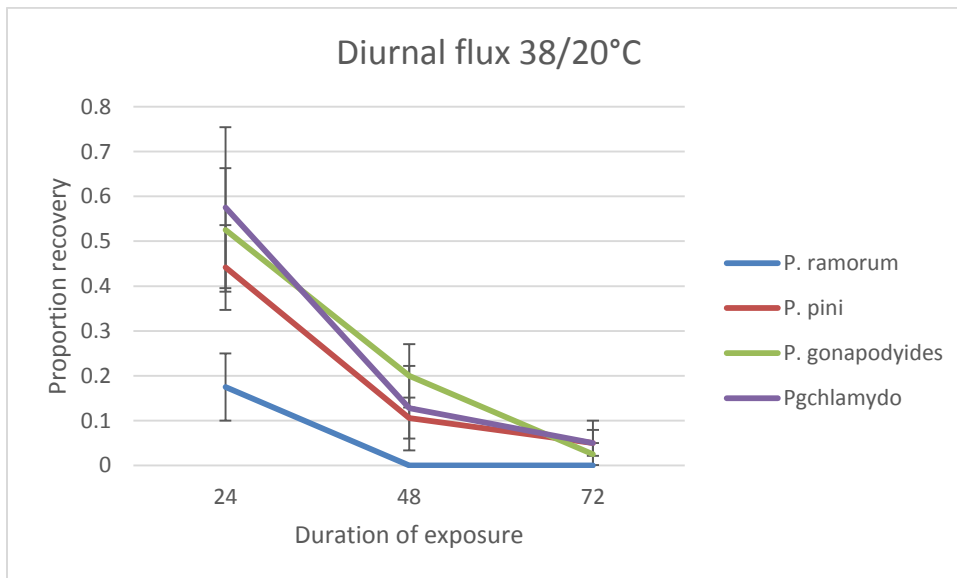


Figure II.2: Recovery of species from diurnal flux between 38 °C and 20 °C



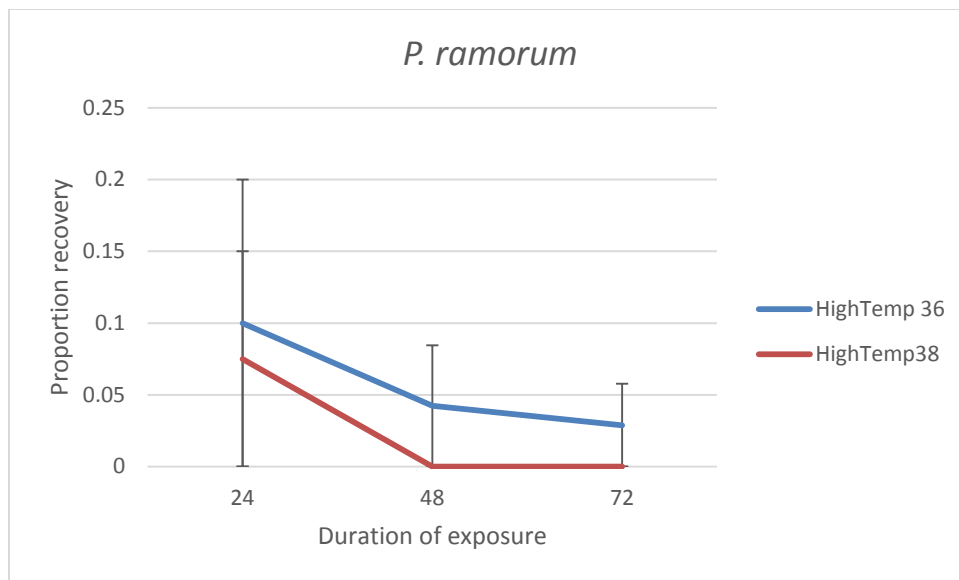


Figure II.3: Comparison of the response of *P. ramorum* samples from two temperature regimes.

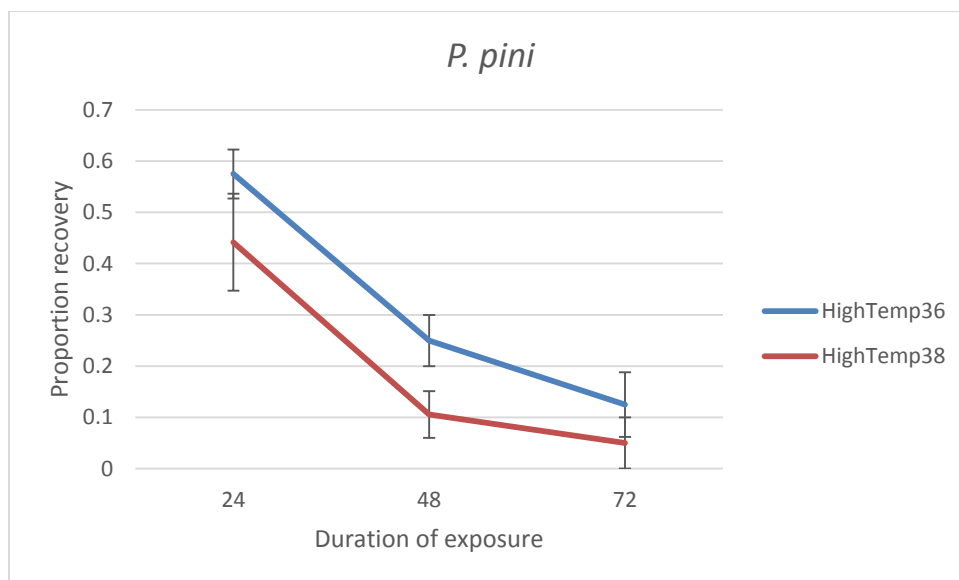


Figure II.4: Comparison of the response of *P. pini* samples from two temperature regimes

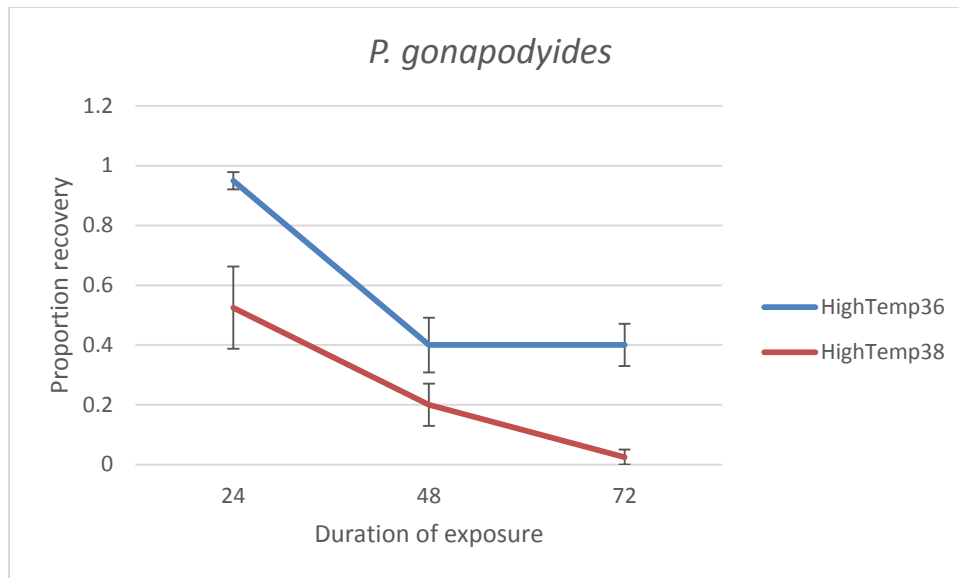


Figure II.5: Comparison of the response of *P. gonapodyides* samples from two temperature regimes

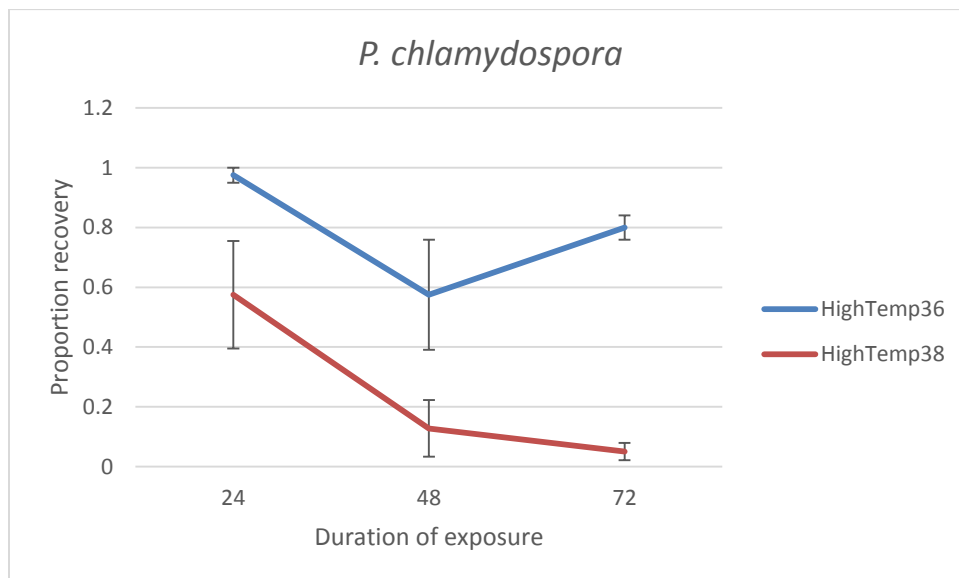


Figure II.6: Comparison of the response of *P. chlamydospora* samples from two temperature regimes