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Progress Report
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Title: Growth chamber studies to determine time and temperature requirements for pathogen and weed seed thermal death

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Executive summary: The objectives of this project were to determine the time and temperature requirements for thermal death of weed seeds and plant pathogens important to the nursery industry. This information is needed to predict the feasibility of soil solarization in killing certain weed seeds and soilborne pathogens problematic in Oregon nurseries. Funds were requested to purchase a high temperature incubator, provide student labor, supplies, and pay for computer programming. We conducted controlled environment experiments with four weed species and five plant pathogen species to estimate the duration of exposure to high temperatures that would be lethal to each species. We then incorporated our findings for four of the species (annual bluegrass, Pennsylvania smartweed, common purslane, and crown gall) into the online soil solarization predictive model https://uspest.org/soil/solarizeV2beta1. Growers in western Oregon, western Washington, and California can now select their location and start date for solarizing soil, and predict how long it will take to kill these target pest(s) at a given soil depth.

Introduction: Soil solarization is a cost-effective and environmentally friendly method for managing soilborne pathogens and weeds. It consists of covering moist, fallow ground with clear plastic film, and allowing solar energy to heat the soil to kill pathogen spores and weed seeds. In previous work funded by the ODA Nursery Research Program and Western SARE, soil solarization of field production beds for 3-9 weeks during the summer before planting demonstrated the effectiveness of soil solarization for reducing populations of *Fusarium, Pythium*, many weed species, and increasing tree seedling growth and stand density (Parke and Mallory-Smith, unpublished). However, the temperatures and time required to kill individual weed and pathogen species differ. The previous online model http://uspest.org/soil/solarize predicted the effectiveness of soil solarization only on two *Phytophthora* species.

Project objective: The objectives of this project were to determine the time and temperature requirements for thermal death of weeds and additional pathogens important to the nursery industry.

Materials and Methods: Seeds of four weed species and propagules of five plant pathogens were included in controlled environment studies evaluating mortality in response to sustained high temperatures.

Table 1. Weed seed species and plant pathogen species tested for thermotolerance under controlled environment conditions at stable temperatures.

Pest	Genus and species	Common name or associated disease	Temperatures tested
Weeds	*Poa annua*	Annual bluegrass	45, 50, 55 °C
	Polygonum pensylvanicum	Pennsylvania smartweed	45, 50, 55 °C
	Amaranthus retroflexus	Redroot pigweed	45, 50, 55 °C
	Portulaca oleracea	Common purslane	45, 50, 55 °C
Pathogens	*Fusarium oxysporum*	Fusarium root rot	45, 50, 55 °C
	Phomopsis sp.	Phomopsis stem canker	45, 50, 55 °C
	Pythium ultimum	Pythium damping-off	45, 50, 55 °C
	Ilyonectria sp.	Cylindrocarpon root rot	45, 50, 55 °C
	Agrobacterium tumefaciens	Crown gall	40, 43.5, 45, 50 °C

Weed seeds

Four weed species were included: annual bluegrass, common purslane, Pennsylvania smartweed, and redroot pigweed. Seeds were collected from fields in Benton or Yamhill County, Oregon. Common purslane and Pennsylvania smartweed seeds were planted in the greenhouse to produce seeds for the study. Seeds were stored at room temperature (21ºC) in dry, dark conditions until use. Germination of the stored seeds was tested before each trial to ensure seeds were viable and nondormant (data not shown). One experimental unit was a sealed capsule (Meter Group, Pullman, WA; 3.9 x 1.1 cm diameter) containing 25 seeds of a species on moist blotter paper (Anchor Paper Corporation, Saint Paul, Mn; Steel blue blotter). Seeds were imbibed 4 h before the heat treatment by saturating the paper with 1 ml deionized water.

Heat treatments. Three temperature treatments, 45, 50, 55ºC, were chosen from the range of reported temperatures in the top 5 cm of the soil profile during the solarization field trials in the PNW (Table 3-2, Wada 2019). The capsules enclosing the imbibed seeds were incubated at constant temperature treatments. The sampling time interval varied from 0.5 h to 24 h based on the sensitivity of a species to each temperature treatment. The incubation trial was continued until a species reached 100% seed mortality for each temperature or up to 336 h. Deionized water was added as necessary to maintain similar moisture levels within the capsules for incubation trials that lasted more than 7 d. The experiments were repeated with three replications for each treatment and included controls which were stored at room temperature (21ºC) in dry and dark conditions.

Seed viability assessment. Any seeds that germinated during the treatments were counted as dead seedlings and discarded based on the apparent physical damage and browning of seedlings. Intact seeds were assessed using the tetrazolium staining method (Patil & Dadlani, 2009) to confirm whether seeds were dormant or dead. Seed coats were partially removed or pierced with a fine needle and soaked in 1% triphenyl tetrazolium chloride solution (Sigma-Aldrich, St. Louis, MO). Seeds were incubated at 30 ºC for 6 hours in the dark for annual bluegrass and 10 hours for other species. The embryo was exposed under a dissection microscope and confirmed viable when the embryo stained red and had no deformation or fungal infection.

Statistical analysis. The experiment was a completely randomized design with both temperature and duration as independent variables. Seed viability was a dependent variable to the treatment. Based on Levine's test for homogeneity of variance ($p > 0.005$), the viable seed data of two trials

for each species of the same treatment and duration were pooled and analyzed as 6 replications. Mortality data were analyzed using the DRC package on R (version 3.5.2) and fitted to the 2-parameter Weibull model defined as follows:

$$v = 100e^{-e^{b\,[\log(d)-a]}} \quad [1]$$

Where v is percent viability of seeds, b is the slope of the curve, the parameter d is a duration of the treatment in hours, and the parameter a is a duration of the treatment in hours where the inflection point of the viability curve is located. The upper limit of seed viability was fixed to 100%, and the lower limit to 0%. For each temperature treatment, parameter estimates, LD90, and their 95% confidence intervals were determined using the summary and estimated effective dose functions.

Plant pathogens

Fungi were isolated from diseased Mazzard cherry seedlings from non-solarized plots at a Clackamas Co. nursery site (collected by J. Parke on June 2, 2017). Oomycete isolates were obtained from dilution plating soil from a nearby field at the same site in 2015 onto PARP selective media. *Agrobacterium tumefaciens* was obtained from the culture collection of Walt Ream, Dept. of Microbiology, OSU. Additional methodology for this pathogen will be added in the final report.

Isolate Identification. All fungal and oomycete isolates were identified using Sanger sequencing. DNA sequence data were trimmed and queried against the NCBI database for species identification. Following molecular identification, isolates of *Pythium ultimum*, *Fusarium oxysporum, Phomopsis* sp. and *Ilyonectria* sp. (Table 4.1, Weidman 2019) were selected for controlled environment trials using wheat seed inoculum. While the species (*Pythium ultimum*, *Fusarium oxysporum)* or genera *(Phomopsis* sp. and *Ilyonectria* sp.) are known to include plant pathogens, the pathogenicity of isolates was not specifically tested.

Inoculum Production. Wheat seeds (organic hard red spring wheat berries, Williams Hudson Bay Farm, Touchet, WA) were mixed with deionized water in a 1:1 volume ratio in Erlenmeyer flasks which were sealed with foam plugs covered with aluminum foil and autoclaved at 121°C for one hour twice at a 24 h interval. After the seeds cooled to room temperature, mycelial plugs of actively growing cultures were mixed in, and the inoculum was incubated at room temperature (19–21°C) in the dark for 14 days.

Temperature Assays. Ten grains of inoculated wheat seeds were placed on water-saturated germination paper (seed germination blotter, Anchor Paper, Saint Paul, MN) inside 12.6 cm^3 lidded plastic capsules (Aqualab, Decagon Devices, Pullman, Washington). Three incubators (Model I36NLC8, Percival Scientific, Inc., Perry, IA) were set to constant temperatures of 45°C, 50°C, and 55°C. Capsules containing inoculum of the different pathogen isolates were placed in each incubator, with periodic removal in replicates of three capsules per exposure period. The specific exposure periods differed among pathogen species. After removal from high temperature incubators, capsules were stored at 4°C for up to 48 h until inoculum was removed and plated onto selective media. Inocula of *Pythium* species were plated onto PARP medium and inoculum of *Fusarium oxysporum*, *Ilyonectria* sp*.,* and *Phomopsis* sp. were plated onto Komada's medium. After 3 days for *Pythium* species or 7 days for fungal species, inoculum survival was assessed by scoring each grain for hyphal outgrowth into the medium. The wheat seeds that resulted in hyphal outgrowth were considered "recovered." The number of recovered seeds relative to the total number of seeds in each capsule were recorded for each treatment.

Statistical Analysis and Predictive Model. All data were analyzed using R statistical software version 3.5.1 (R Core Team, 2017). Survival data were fit to a probit logistic regression model for each trial, with survival as the response and duration and trial number as the explanatory variables. The family was set to quasi-binomial to account for overdispersion of data. Trials were combined into a single dataset when the interaction between trial and duration was not significant (α=0.05). The model was used to calculate the dose requirement of temperature and duration necessary to eliminate 99.9% of propagules ($LD_{99.9}$) for each pathogen.

Results: Results of thermal tolerance of weed seeds are shown in Table 2 and Figures 1-4. Results for fungal and oomycete plant pathogens are shown in Table 3 and Figures 5-8. Results for the bacterial plant pathogen, *Agrobacterium tumefaciens*, are shown in Figure 9.

Table 2. Durations (hours) necessary to kill 90.0% ($LD_{90.0}$) of weed seeds at given temperatures during constant exposure. $LD_{90.0}$ estimates ± standard error are indicated. Data are from Wada (2019).

Weed species	45 °C	50 °C	55 °C
Annual bluegrass	47 ± 0.9 h	7 ± 0.1 h	1 ± 0 h
Common purslane	3246 ± 2164 h	829 ± 75.1 h	41 ± 1.7 h
Pennsylvania smartweed	124 ± 3.8 h	115 ± 5.1 h	3 ± 0.1 h
Redroot pigweed	251 ± 4h	88 ± 2.7 h	5 ± 020 h

Table 3. Durations (hours or minutes) necessary to eliminate 99.9% ($LD_{99.9}$) of different plant pathogens at given temperatures during constant exposure. $LD_{99.9}$ estimates ± standard error are indicated. Data are from Weidman (2019).

Pathogen species	45 °C	50 °C	55 °C
Fusarium oxysporum	65.2 ± 3.4 h	14.4 ± 0.8 h	4.6 ± 1.3 h
Phomopsis sp.	582.5 ± 32.0 min	97.2 ± 3.8 min	38.4 ± 1.9 min
Ilyonectria sp.	69.0 ± 4.5 min	32.1 ± 131.4 min	23.7 ± 267.6 min
Pythium ultimum	81.1 ± 5.8 min	35.6 ± 1.2 min	16.6 ± 0.0 min

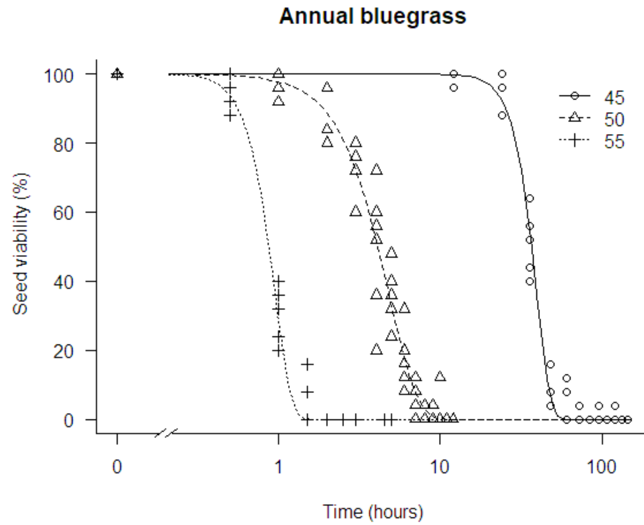

Figure 1. Thermal dose-response curve of annual bluegrass. Annual bluegrass percent viability vs. time at constant temperature treatments. Seed viability at each sampling event at 45 (○), 50 (△), and 55(+) ºC.

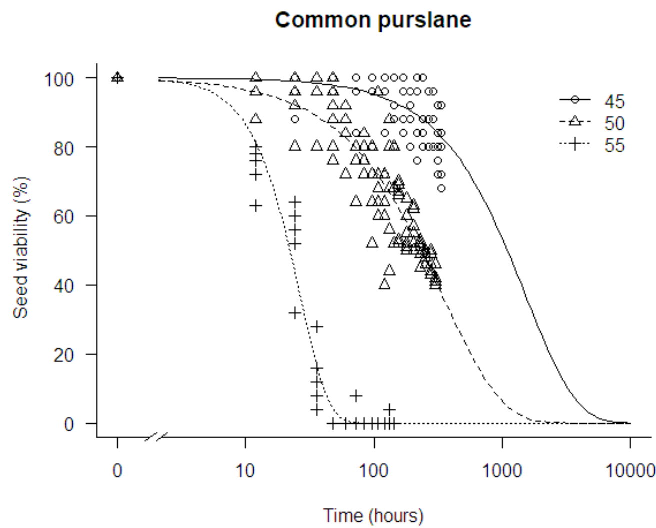

Figure 2. Thermal dose-response curve of common purslane. Common purslane percent viability vs. time at constant temperature treatments. Seed viability at each sampling event at 45 (○), 50 (△), and 55(+) ºC.

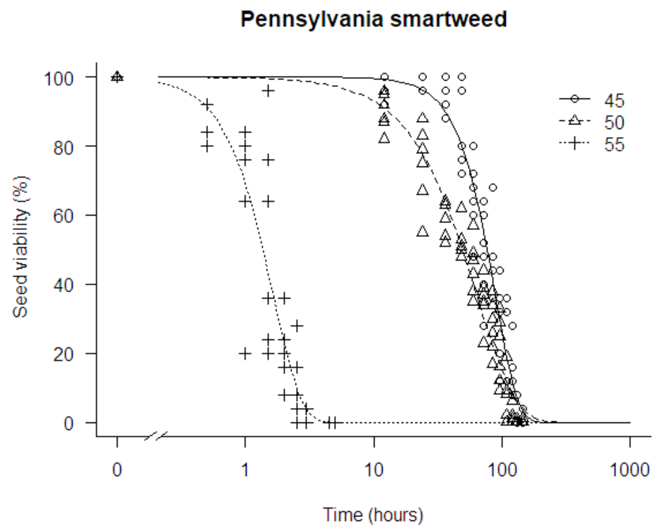

Figure 3. Thermal dose-response curve of Pennsylvania smartweed. Pennsylvania smartweed percent viability vs. time at constant temperature treatments. Seed viability at each sampling event at 45 (○), 50 (△), and 55(+) ºC.

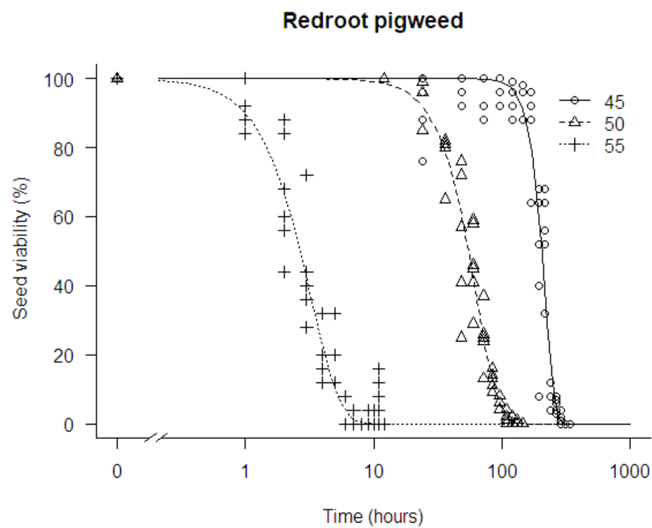

Figure 4. Thermal dose-response curve of redroot pigweed. Redroot pigweed percent viability vs. time at constant temperature treatments. Seed viability at each sampling event at 45 (○), 50 (△), and 55(+) ºC. At 45 ºC.

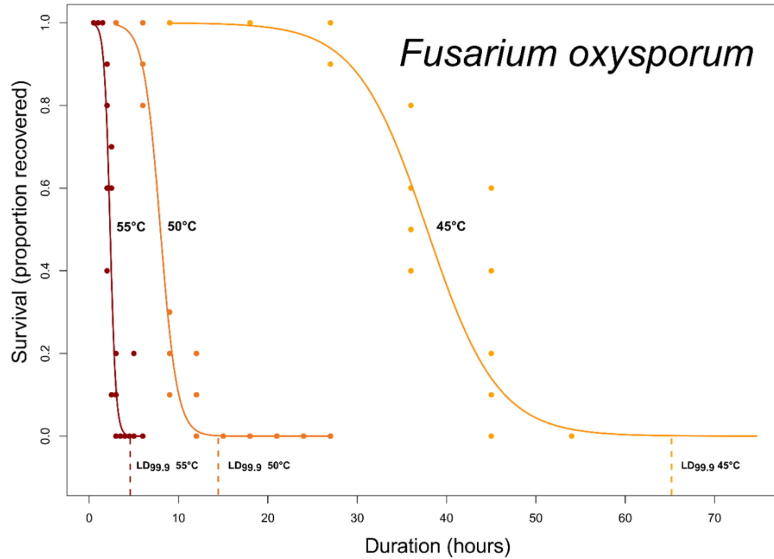

Figure 5. *F. oxysporum* response to heating at 45, 50, 55 °C for different durations. Estimated duration of heating (in hours) expected to kill 99.9% ($LD_{99.9}$) of this isolate at each temperature are designated by dotted vertical lines which intersect with x-axis.

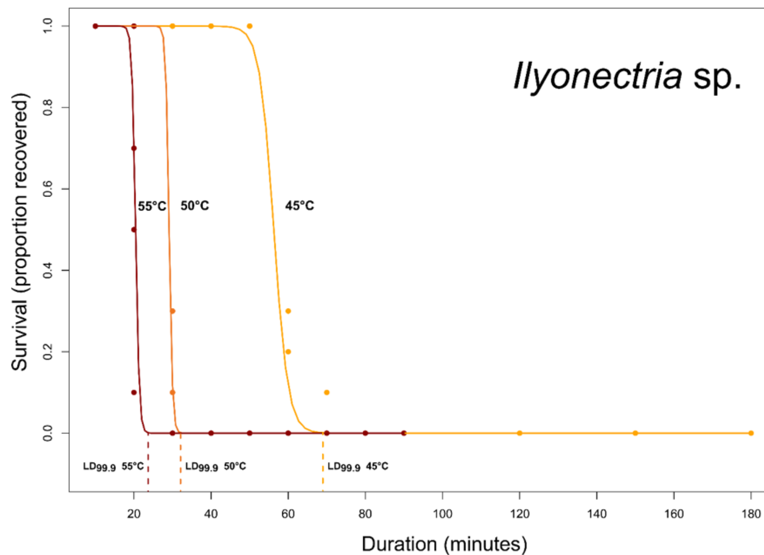

Figure 6. *Ilyonectria* sp. response to heating at 45, 50, 55 °C for different durations. Estimated duration of heating (in minutes) expected to kill 99.9% ($LD_{99.9}$) of this isolate at each temperature are designated by dotted vertical lines which intersect with x-axis.

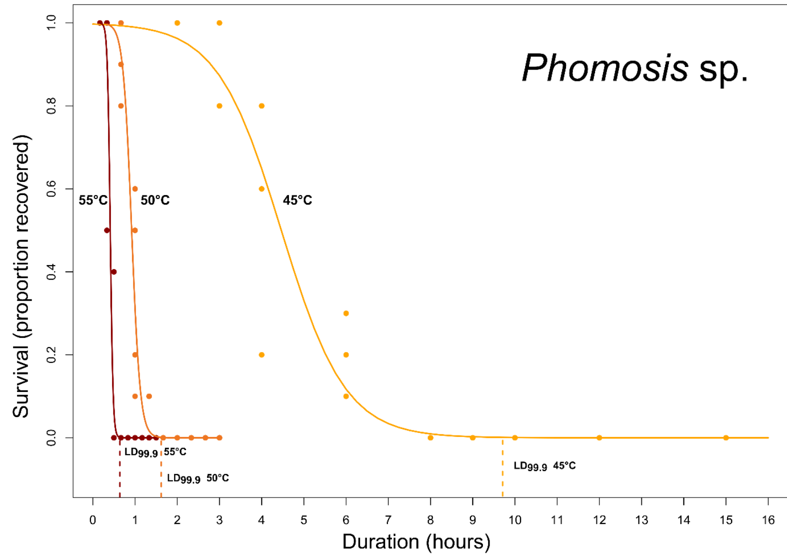

Figure 7. *Phomopsis sp.* response to heating at 45, 50, 55 °C for different durations. Estimated duration of heating (in hours) expected to kill 99.9% ($LD_{99.9}$) of this isolate at each temperature are designated by dotted vertical lines which intersect with x-axis.

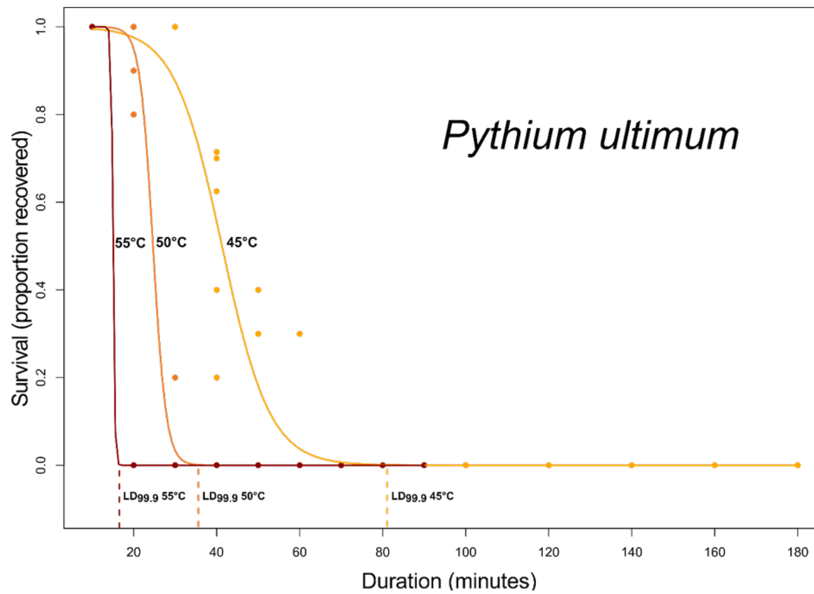

Figure 8. *Pythium ultimum* response to heating at 45, 50, 55 °C for different durations. Estimated duration of heating (in minutes) expected to kill 99.9% ($LD_{99.9}$) of this isolate at each temperature are designated by dotted vertical lines which intersect with x-axis.

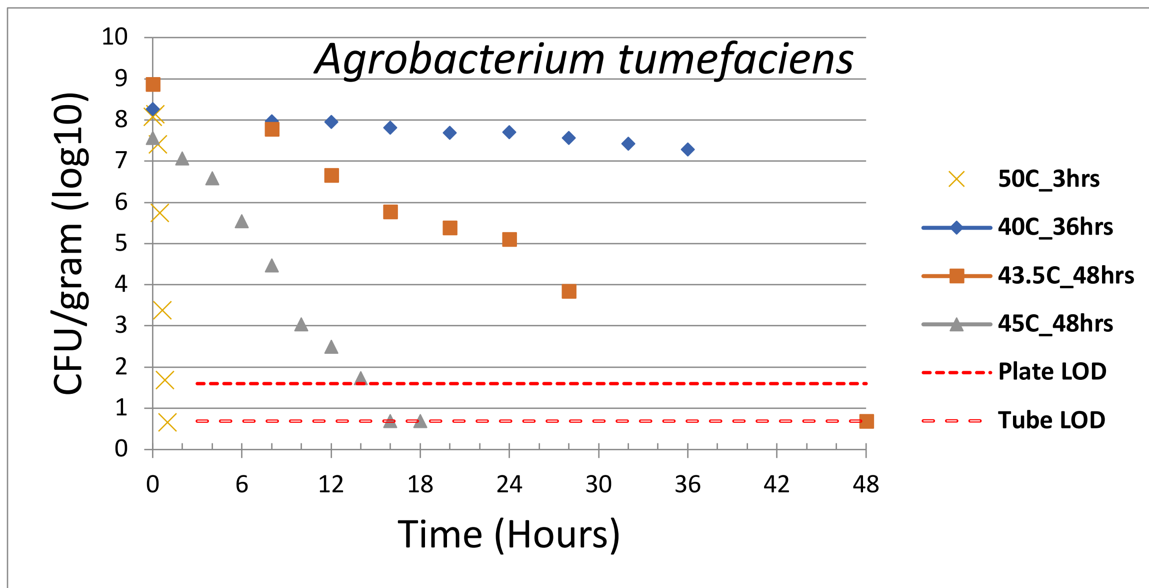

Figure 9. *Agrobacterium tumefaciens* response to heating at 40, 43.5, 45, and 50 °C for different durations. CFU = colony-forming units per gram of sand. Limit of detection with culture plates and tubes is indicated by dashed red lines.

Discussion

Results from the controlled environment experiments suggest that temperatures typically achieved during a solarization during the summer months in Oregon will be sufficient to kill seeds of annual bluegrass in the top 5 cm of soil. Pennsylvania smartweed, and redroot pigweed may be killed to a depth of 5 cm in some but not all years. It is unlikely that soil solarization will kill seeds of common purslane except for those at or very close to the soil surface, where soil temperatures are hottest. The critical depth at which seeds of each species cannot successfully emerge will determine the depth at which exposure to high soil temperatures from solarizing is effective. Soil temperatures during solarization will in some cases also be sufficient to reduce the viability of seeds in the weed seedbank, leading to reduced weed pressure in subsequent years.

Most of the plant pathogen species tested were generally more sensitive to high temperatures than were the weed seeds. *Fusarium oxysporum* was the most tolerant to high temperatures, requiring 4.6 hours above 55 °C, whereas the other fungal species were killed by periods of less than one hour at 55 °C. It is very likely that populations of soilborne pathogens in top few cm of soil will be killed or drastically reduced by soil solarization.

Growers may now go to the online soil solarization predictive model (beta version) https://uspest.org/soil/solarizeV2beta1 to predict the effectiveness of soil solarization for several target species of interest (weeds and plant pathogens). By selecting the location and start date for solarizing, the model will predict the soil depth at which solarization will kill the target species, and determine the length of time necessary to solarize. Growers may use this online model to plan the shortest and most effective period to solarize.

Benefit to nursery industry: Cultural practices that result in fewer weeds, improved soil quality and healthier trees increase both economic profitability and environmental sustainability of Oregon nurseries. Moreover, use of soil solarization results in a dramatic reduction in labor

needed for handweeding. Results at two Oregon nurseries indicate that handweeding time in solarized beds is reduced by approximately 50% relative to non-solarized beds. Labor costs and labor shortages are one of the most serious limitations currently facing the nursery industry (Digger, May 2016). Increased seedling stand density resulting from a reduction in the population of damping-off pathogens with solarization would also potentially allow lower seeding rates and thus lower seed costs. However, nurseries need accurate information to decide whether soil solarization will be effective against the spectrum of weeds and soilborne pathogens that are problematic at their sites, and what durations of soil solarization will be required. Data obtained from these growth chamber studies has provided information needed to expand our current predictive model to target several more pathogen and weed species so that soil solarization can be implemented more widely.

References

Wada, N. 2019. Soil solarization for weed management in tree seedling nurseries in the Pacific Northwest. M.S. Thesis, Oregon State University. 98 pp.

Weidman, C. 2019. Soil solarization in Pacific Northwest tree seedling nurseries: effects on soilborne plant pathogens, soil nutrients and the soil microbiome. M.S. Thesis, Oregon State University. 254 pp.