Can thermotherapy be used to eliminate *Phytophthora* from infected root systems of container plants?

Final report for task 3 of:

An experimental management project to protect against the sudden oak death and other plant pathogens in SFPUC watersheds

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Cover photo: *Quercus agrifolia* plants 11 days after heat treatment at 47 C for 30 minutes about 1 month after inoculation with *Phytophthora kelmanii*, *P. cambivora*, and *P. cactorum* (left to right).

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Summary

We conducted a pilot study to investigate the feasibility of using heat to eradicate *Phytophthora* root infections in living container-grown plants. Based on our review of available literature, it appeared that temperatures between 34 and 51 C for various treatment times might be usable for thermotherapy. We conducted a series of plant thermotolerance tests using various container-grown California native plant species. Some treatment temperature × time combinations killed plants or caused unacceptable damage, but 47 C for 30 minutes resulted in minimal damage. *Phytophthora cactorum*, *P. kelmanii*, and *P. cambivora* in infected pear epidermis or rhododendron leaves could be rendered nonviable when held in 47 C water for 20 minutes or longer. A 30-minute treatment at 47 C appeared to eradicate these 3 species from inoculated *Quercus agrifolia* and *Q. lobata* grown in small (397 ml) containers. No *Phytophthora* was recovered by leachate baiting after 11-12 days, 22-28 days, or 43 days after treatment or by direct root system baiting at the end of the study 82 to 93 days after heat treatment. Instrumentation of heat-treated plants and inoculum was essential for accurate treatment. Heat transfer into plants and inoculum placed in a heated water bath was slow. Unless materials were preheated with hot water to target temperatures in advance, the temperature × time of the heat-treated materials was always less than the temperature × time that the material was in the heated water bath.

Introduction

Plant thermotherapy involves heating plant parts or entire plants to temperatures capable of killing or inactivating internal pathogens. Thermotherapy can work as a control measure if the plant can tolerate greater temperatures than the target pathogen. The use of thermotherapy dates to at least the mid-1800s and the first scientific reports came in the late 1800s (Jensen 1887, 1888). Heat treatment has commonly been applied to eliminate pathogens from dormant propagative material, most commonly seeds and vegetative propagules. Another common application of thermotherapy has been to free woody plants from viruses (e.g., Wood 1973) and recent work has been done on in-field treatment of HLB-infected citrus trees with steam (Ehsani 2016).

Some limited work has been done using thermotherapy to treat plants with *Phytophthora* root infections (e.g., Benson 1978). *Phytophthora* is a promising target organism for thermotherapy because many species can be killed by exposure to temperatures of 49-52 C for about 30 minutes. In some of our *Phytophthora* experiments at UC Davis, we detected soil temperatures of at least 42 C in container nursery plants in mid to late summer. We also observed that after exposure to high summer temperatures, including high irrigation water temperatures, recovery of *Phytophthora* from some documented *Phytophthora*-infected plants became erratic, suggesting that *Phytophthora* inoculum levels in these container plants may have been reduced by the excessive heat.

California native plants are potentially good targets for use of thermotherapy because many of these species are normally exposed to high temperatures during the growing season, suggesting that they may tolerate heat treatments. In this project, we conducted a literature review to identify possible parameters for thermotherapy treatments. We then conducted a series of experiments testing the heat sensitivity of various container-grown California native plants and *Phytophthora* inoculum. The project concluded with a trial testing whether *Phytophthora* infection could be eradicated from roots of live plants.

Literature review

Sensitivity of *Phytophthora* species to heat

We reviewed scientific literature to help identify temperature parameters that were likely to be useful for thermotherapy of *Phytophthora*-infected plants. The first component of this was determining the minimum temperature regimes needed to kill *Phytophthora* species. Comparing the studies on this topic was difficult because of differences in methods and temperatures used. Furthermore, individual studies tested only a few time × temperature combinations, leaving many unanswered questions as to whether shorter time intervals or lower temperatures might have been effective.

Data from several pertinent studies are summarized in Table 1. Among species tested in these studies, *Phytophthora ramorum, P. cactorum,* and *P. cinnamomi* appeared to be the least tolerant of high temperatures. In contrast, *P. nicotianae* and *P. pini* appeared to be much more resistant to heat. For example, *Phytophthora* in infected rhododendron leaf discs in water held in temperature chambers were killed in 0.33 hours at 50 C for *P. ramorum* and in 1.19 hour for *P. pini* (Funahashi and Parke 2018). Large differences in heat tolerance for these two species were also noted in experiments run at lower temperatures (Table 1). For *P. nicotianae,* 24 hours at 42 C killed an aqueous suspension of chlamydospores (Hao et al. 2012). In a different study, *P. nicotianae* chlamydospores in soil were killed after 96 hours at 41 C or 48 hours at 44 C (Coehlo et al. 2000).

Studies in which propagules were directly exposed to hot water generally required shorter heat exposure periods to attain thermal kill than studies in which the propagules were placed in soil in a container that was placed in hot water. Based on our experience (discussed below), we believe that this is likely to be an artifact of the incubation system. Most studies we reviewed did not directly measure the temperature of the heated inoculum or infested material. Instead, the amount of time that samples were introduced or removed from the heating device is typically reported.

However, a variety of factors, including rates of heat transfer and evaporative cooling from water surfaces can affect whether the treated material reaches the temperature of the heated environment and how quickly temperature equilibration occurs. Also, close examination of the methods shows none of the experimental systems used in the papers summarized in Table 1 accounted for the cooling that occurs when the inoculum was added to the containers used in the heat treatment systems. Without direct measurements of the temperature of the treated inoculum, it is not possible to know when or if the propagules reached the stated incubation temperature. Thus, in many studies, reported times required to kill *Phytophthora* inoculum at various temperatures may overestimate the actual value.

Plant thermotolerance

We also reviewed studies that reported plant survival after various heat treatments (Table 2). Three-year-old potted citrus trees were able to survive 7 days in a growth chamber at a constant temperature of 42 C (Hoffman et al 2013). Potted periwinkle (*Catharanthus roseus*) also survived this temperature regime.

Bare root strawberry plants survived immersion in 48 C water for 1 hour (50% survival) when placed directly in hot water (Turechek and Peres 2009). However, if they were first sealed in plastic bags before being placed in 48 C water, they had 50% survival after 2 hours. However, no data were presented to verify the temperatures that plants in the sealed bags experienced over this interval.

Methods for heat-treating plant roots

Hot air or hot water are most commonly used for plant thermotherapy. For hot air treatments, the entire plant is normally maintained in a heated chamber. One disadvantage of this method is that the entire plant is exposed to the high temperatures, which may not be tolerated well by shoots or leaves. Long-term hot air treatments can also cause water stress in addition to heat stress. If water has to be added to prevent shoot desiccation, the applied water is likely to change the soil temperature, complicating the temperature regime. Evaporative loss of water from the soil can reduce soil temperature below the ambient temperature in the chamber.

In contrast, hot water can be selectively applied to the root system and can potentially provide for more precise temperature control. However, direct immersion of plants in a shared water bath would allow cross contamination to occur if the heat treatment was not effective. Hot water treatment also exposes root systems to saturated conditions that can become increasingly anoxic over time. These conditions can damage root tissues on their own, particularly if the treatments continue for many hours or days, and detrimental effects may be more pronounced at high temperatures.

Table 1. Hours of heat needed to kill *Phytophthora* propagules in various studies. Effective treatment times are highlighted in pink, ineffective time in green.

		Hours at temperature for thermal kill (closest listed temperature)											
	C	**37**	**38.5**	**39.1**	**41**	**42**	**44**	**45**	**47**	**48**	**50**	**51**	**60**
Ref	**F**	**97**	**101**	**102**	**106**	**108**	**111**	**113**	**117**	**118**	**122**	**124**	**140**
1	*Pythium ultimum* – culture on PDA	480	312			45		9	3.3		0.55		
2	*P. nicotianae* – chlamydospores in soil				96		48		4				
3	*P. nicotianae* – aqueous suspension of chlamydospores					24				6			
2	*P. nicotianae* – chlamydospores in soil; multiday: 3 or 5 h at high temperature, cycling temp back to 25 C						Still live after 5 h x 15d		3 h x 3d				
4	*P. ramorum* – infected rhododendron leaf discs in water										0.33		
4	P. *ramorum* – infected rhododendron leaf discs in soil; multiday: ramp up from 26 C to 42 C then back down					2.5 h x 6 days							
3	*P. pini* – aqueous suspension of oospores					12				6			
4	*P. pini* -infected rhododendron leaf discs in water										1.19		
4	*P. pini* – infected rhododendron leaf discs in soil; multiday: ramp up from 26 C to 42 C then back down					2.5 h x 10d							
5	*P. cactorum* –oospores in soil and colonized walnut twigs							0.5					
5	*P. cinnamomi* – chlamydospores in soil and colonized walnut twigs							0.33					
6	*P. cinnamomi* – mycelium		2										
6	*P. cinnamomi* – mycelium + chlamydospores				2								
References: 1= Pullman et al. 1981, 2=Coelho et al. 2000, 3= Hao et al. 2012, 4=Funahashi and Parke 2018, 5=Juarez-Palacios et al. 1991, 6=Gallo et al. 2007													

Table 2. Heat tolerance of various plants in several studies. Highlighting indicates whether temperatures were tolerated (green) or not (pink) by test plants.

Hours at temperature									
	C	**42**	**44**	**45**	**47**	**48**	**50**	**51**	**60**
Reference	**F**	**108**	**111**	**113**	**117**	**118**	**122**	**124**	**140**
Turechek and Peres 2009	strawberries in water bath		4 (~50% survival)	4 (~50% survival)					
Turechek and Peres 2009	strawberries in plastic bags in water bath		4			2 (~50% survival)		0.1 h killed	
Chester et al 1957	*Gladiolus* cormels dry heat						1.5		not tolerated
Chester et al 1957	*Gladiolus* cormels water bath						0.5		not tolerated
Hoffman et al 2013	3 year old potted citrus trees dry heat. Periwinkle (*Catharanthus roseus*) also survived 42 C constant temp for 7 days.	168 h, (7 days) some damage but recovered		16 h for 6 days cycled down to 30C (86F) for 8 h/day					

Experimental thermotherapy parameters used

Based on data that *P. pini* required 1.19 hours for thermal death in rhododendron leaf discs in hot water (Funahashi and Parke 2018, Table 1), we hypothesized that treatment of 1.5 hours at 50 C would be needed to kill *Phytophthora* in an infected plant, allowing for some margin of safety. However, we did not find any reports indicating whether plants could survive this regime. Gladiolus cormels survived this time × temperature when dry heat was used, but only tolerated 0.5 h at 50 C in a water bath (Table 2). It was therefore necessary to conduct a series of plant thermotolerance tests to find temperature × time combinations that various native plants grown in containers could survive that were also likely to kill *Phytophthora*.

Based on findings from the initial plant thermotolerance tests discussed below, the time × temperature combinations that plants might be able to tolerate were generally lower than 1.5 h at 50 C. Therefore, we conducted additional *Phytophthora* thermotolerance tests because such data were lacking in the temperature range of interest. Finally, we conducted thermotherapy tests in which infected plants were heat-treated and subsequently tested for inoculum production and plant survival. The details of these studies are presented below.

Methods

Water bath construction

We constructed water baths from readily obtainable materials that would be applicable to use in nurseries. Hot water treatments were conducted using plastic bins. The bins were wrapped on the outside with insulating material and placed on a sheet of polystyrene insulation to minimize heat loss. We used an electric water heating element controlled with a digital thermostat (Inkbird ITC-308) to maintain the water temperature in the target range. For tests 1 and 2, the water was heated by a 100 W aquarium heater (Penn-plax Therma-flow "PC") in a shallow rectangular bin (46 ×33 cm, 20 cm deep). For all subsequent tests, a 1000 W bucket heater (Gesail Model 05-742G) was used to heat the water in a deeper square plastic bin (39 ×39 cm [bottom], 55 cm deep). A small electric fountain pump placed near the heating element was used to continuously circulate water in the bin during each test. The pump had a flow rate of about 3.8 L/min.

Our first experiments involved the technical aspects of treating plants at the desired temperature × time regimes and assessing plant tolerance for these regimes. Hot water treatments were conducted indoors for tests 1 and 2 using the shallower rectangular bin. Subsequent tests were conducted outdoors in the deeper square bin, which was covered during treatment to slow heat loss. The bins were filled with water to a level that was just above the top of the potting media in the container plants. The volume of water varied between tests based on the position and number of plants, but the volume of water was kept to the minimum needed to allow for adequate circulation and temperature distribution.

1. Plant thermotolerance experiments

Plants were provided by Grassroots Ecology Nursery in January 2020 (Table 3). Plants had been grown in heat-treated soil following Nursery *Phytophthora* BMPs but were in a holding area for excess plants and had not been tested recently. Plants were in tapered square plastic containers: AB35 containers were 12.7 cm (5 inches) tall and 6 cm (2.4 inches) wide, volume 397 ml; AB46

containers were 15.2 cm (6 inches) tall and 9.2 cm (3.6 inches) wide, volume 1098 ml. Some of the plants were completely dormant, and most were not actively growing due to low winter temperatures and light levels. All plants except the *Juncus xiphioides*, *Lonicera hispida,* and *Rubus ursinus* had been cut back at some point during production.

Multiple temperatures loggers (calibrated Inkbird THC-4 and Elitech RC-4 with external temperature probes) were used to monitor soil temperatures at 10-minute intervals during the tests. Temperature logger probes were placed directly into the potting media either in the center of containers (to record temperature at coolest portion of soil mass) or at the outer edge against the inside of the container (to record temperature at the hottest portion of the soil mass). Probes were placed vertically with the tip of the probe about 6 cm below the soil surface. Loggers were placed in pots adjacent to and far from the heating element to check for temperature uniformity throughout the water bath. In test 3, one logger probe was inserted horizontally in the top 1 cm of the potting media to monitor temperature at the soil surface.

In all tests, pots of treated plants were individually bagged in plastic bags. Leaks sometimes developed because rough edges of pots created small holes in the bags. Tops of the bags were kept open to allow for air exchange. As noted below, water was intentionally or unintentionally introduced into the bags for some of the plants.

Wrapped containers were buoyant, so it was necessary to devise a way to keep the containers submerged in the heated water. For test 1, stiff wire mesh was used to hold the surface of the pots even with the water surface, but the containers tended to tip over and some needed to be righted during the test. For subsequent tests, the bagged containers were tied down to a metal rack with nylon twine and the rack was held down using L-shaped PVC pipe sections clamped to the side of the bin.

Table 3. Species obtained from Grassroots Ecology Nursery used in heat tolerance experiments. Target temperatures for each test are listed, actual temperatures achieved are discussed below.

		Number of containers / number flooded containers		
Species	**Container size**	**Test 1 45 C × 34 h**	**Test 2 41 C × 96 h**	**Test 3 50 C for 1.5 h**
Cornus sericea	AB35	2	2	
Juncus xiphioides	AB35	3	3 / 2 flooded	6 / 3 flooded
Lonicera hispida	AB35	3	3 / 1 flooded	
Rubus ursinus	AB35	3	3 / 1 flooded	
Symphoricarpus albus	AB35	3	3 / 2 flooded	
Ribes sanguineum	AB46	2	2 / 1 flooded	2 / 2 flooded
Rubus parviflorus	AB46	2	2 / 1 flooded	2 / 2 flooded
Total containers		18 / 0 flooded	18 / 8 flooded	10 / 7 flooded

Test 1 – Target 45 C for 34 h

Our initial plan for test 1 was to heat the root systems of the plants to 50 C for 1.5 hours. Containers were put in the water bath before hot water (52 C) was added. Water cooled as it was added and the 100 W heating element we used could not maintain the water at the initial target temperature, so we lowered the target temperature to 45 C. The treatment time, 34 hours, was

interpolated from a regression line of data points from Coelho et al (2000) for the amount of time needed to kill *Phytophthora nicotianae* chlamydospores in soil at this temperature (Figure 1). Plants were watered before the test and none flooded during the test. Over this long heat treatment, water needed to be added periodically to maintain the appropriate level in the water bath. To avoid cooling of the water bath, added water needed to be about 5 C above the target treatment temperature. Test 1 was started on 1/27/20.

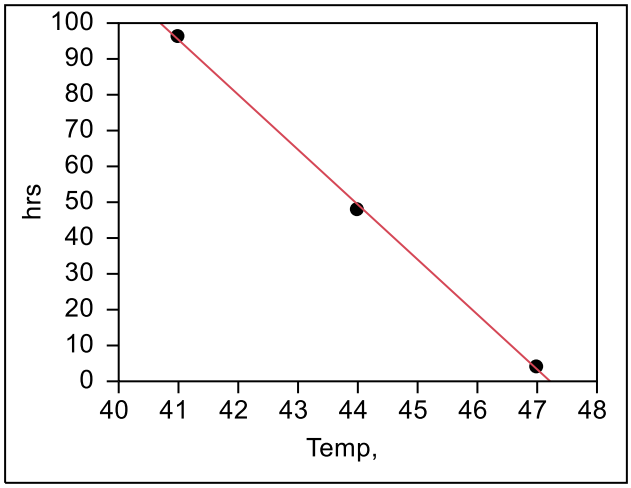

Figure 1. Hours needed to kill chlamydospores of *Phytophthora nicotianae* in soil at various temperatures from data in Coelho et al. (2000).

Test 2 – Target 41 C for 96 h

Target parameters for test 2 were 41 C for 96 hours, based on one of the *Phytophthora nicotianae* thermal kill data points from Coelho et al. (2000) (Table 1, Figure 1). Plants were not initially flooded, but due to leaks that developed in some bags, 7 of the 18 plants flooded during the test. As in test 1, plants were placed in the bath first, and then 45 C water was added. Test 2 was started on 1/29/20.

Test 3 – Target 50 C for 1.5 h

The plan for test 3 was to treat containers at 50 C for 1.5 hours, based on the amount of time needed to kill *P. pini* according to Funahashi and Parke (2018) (Table 1, 1.19 h). We used the 1000 W bucket heater, which heated water very quickly and was able to maintain the desired water temperatures. However, because the bucket heater required a minimum water depth that was greater than could be achieved in the rectangular bin we had been using, the test was carried out in the deeper square plastic bin. The water level in the bin was adjusted to just above the tops of the AB46 containers (about 17 cm) and then the bucket heater was turned on.

In this test, after some plants flooded due to leaks, we intentionally flooded most of the remaining test plants to obtain more uniform heating, particularly in the larger AB46 containers. Half (3 of 6) of the AB35 containers in this test were left unflooded to compare the effects of flooding on this subset. Test 3 was started on 2/3/20.

Test 4A – Target 47 C for 4 h

This and later tests used additional plants grown from seed in AB35 containers according to Nursery *Phytophthora* BMPs by Central Coast Wilds nursery (*Juncus patens, J. xiphioides*) or the authors (*Quercus agrifolia, Q. lobata, Ribes malvaceum*) (Table 4). Prior to start of experiment, plants were held overnight at room temperature and data logger probes were inserted into the center of the rootball area of two containers: a *Q. lobata* and a *J. patens*.

Containers were placed individually in plastic bags. Rubber bands were used to keep bags as tightly wrapped as possible against the outer sides of the containers. Containers were placed in a wire mesh basket and placed several cm deep into the water bath containing 47 C water. The instrumented *Q. lobata* was positioned at the outside edge of the 22 pot array and the instrumented *J. patens* was positioned in the middle of the array. Water heated to 47 C was then added to each pot until soil was flooded. Containers were then lowered all the way into the water bath until the water level was about 1 cm above the container tops. Containers were removed from the water bath after data loggers showed that the soil temperature had been at 47 C for 4 h in at least one of the containers. The test was conducted on 4/22/2020.

Table 4. Plant material used in test 4A.

Species	Container size	Phenology / condition before treatment	Number of containers (all flooded)
Juncus patens	AB35	Top cm of leaves with necrosis/chlorosis, about 20-30% of leaves are dead	2
Juncus xiphioides	AB35	Tip 5mm of leaves with slight chlorosis, about 20% dead leaves	2
Quercus agrifolia	AB35	No active shoot growth, leaves hardened off (last year's leaves)	2
Quercus lobata	AB35	No active shoot growth, new leaves are expanded, soft, not hardened off.	2
Ribes malvaceum	AB35	No active shoot growth, some unexpanded leaves, a few larger leaves chlorotic	2

Figure 2. Water bath setup for tests 4A and B. PVC pipes were used to keep containers submerged. Bucket heater is at upper left. Wires are for dataloggers measuring temperatures and the thermostat.

2. *Phytophthora* heat sensitivity experiments

Test 4B – *Phytophthora* survival in potting media at 47 C for 4 h

Concurrent with test 4A, in the same water bath, we tested whether *Phytophthora* inoculum could survive the 47 C × 4 h heat treatment under the same conditions used to test plant thermotolerance.

Phytophthora isolates shown in Table 5 were used to inoculate green D'Anjou pears, one isolate per pear. These three species can readily be distinguished from each other in culture. Inoculations were performed on surface-disinfested pears (submerged 45 seconds in 0.5% NaOCl) by cutting a small flap (about 7-8 mm) into the pear epidermis and gently peeling it back from the underlying mesocarp. A small (1.5-2 mm^3) block of agar with mycelium from the edge of an actively-growing culture was placed under the flap. A strip of Parafilm was used to hold the flap closed and prevent it from drying out. Fifteen days after inoculation, a cork borer was used to cut 7 mm diameter epidermal disks from around the edges of the *Phytophthora* lesions on the pears. Disks consisted of symptomatic epidermis plus some adhering mesocarp, with a total thickness of about

0.5 mm. Pears were heavily colonized and microscopic examination of *P. cactorum* lesions showed oospores were plentiful in the pear epidermis.

Three disks of each isolate were placed in fine mesh bags (nylon tea bags, folded to 4.5 x 3 cm size) that were buried in heat-treated potting mix in AB35 containers. The folded tea bags were placed in the center of the potting mix in the containers 6 cm from the bottom. Three replicate containers were used for each *Phytophthora* species. A fourth container for each *Phytophthora* species was prepared in the same manner but held at room temperature for the duration of the test.

Containers were placed in individual plastic bags and placed in the 47 C water bath. All containers were removed from the water bath after four hours at 47 C, as indicated by temperature logger readings. After the soil had cooled completely, disks were recovered from both heated and control *Phytophthora* containers and plated on carrot cornmeal agar. One disk was divided in half and one half was plated while the other half was used to inoculate a green D'Anjou pear by placing it under a small flap of pear epidermis as described above.

Inoculated pears were incubated at room temperature and observed for a minimum of 7 days for the development of *Phytophthora* lesions around the inoculation points. Isolations from lesions were made to confirm that the lesions contained the target *Phytophthora* species.

Table 5. *Phytophthora* species used in test 4B.

Species	Abbreviation	Container size	Material tested	Number of containers (all flooded)
P. kelmanii	KEL	AB35	three 7-mm-diam disks from pear lesions per pot	3
P. cambivora	CAM	AB35	three 7-mm-diam disks from pear lesions per pot	3
P. cactorum[1]	CAC3	AB35	three 7-mm-diam disks from pear lesions per pot	3
P. cactorum[2]	CAC4	AB35	three 7-mm-diam disks from pear lesions per pot	3

1- Isolate PR20190822-S1-HEAR01-5 from *Heteromeles arbutifolia*, Alameda Co. = 100% ITS sequence match to *P. cactorum* type
2- Isolate PR20200220-ADOB39-4 from *Frangula californica*, Santa Clara Co. = 100% ITS sequence match to *P. cactorum* haplotype "CAC3" as designated by T. Bourret

Test 5 – Target 47 C for 2 to 3.5 h, *Phytophthora*-infected pear epidermal disks

Because test 4B had shown that 4 hours at 47 C was lethal to the *Phytophthora* isolates tested, in this trial we tested 2, 2.5, 3, and 3.5 h incubation periods. The same four *Phytophthora* isolates listed in Table 5 were used in this test. *Phytophthora*-infected pear epidermal disks were heated in water in test tubes that were placed in a water bath so that the heating times could be controlled more precisely.

A cork borer was used to cut 7 mm diameter disks from the outer edges of D'Anjou pear lesions inoculated 7 days previously as described for test 4B. Oospores were visible in disks infected with *P. cactorum* isolates. Because we expected the shortest treatment times at this temperature to be ineffective based on published literature, we did not include a non-heated control in this test.

Steps were taken to minimize the amount of time required for disks to reach the target temperature. Prior to adding disks, test tubes were fastened in a rack which allowed free water movement on all sides. Two additional test tubes in the rack were used to hold temperature logger probes. Preheated (85 C, 185 F) sterile water (previously boiled) was added to each tube to a height of 8 cm (17 ml), which was about 0.5 cm below the height of the water line in the water bath. This water cooled as it was added. The test tube rack was then lowered into a 47 C water bath.

When temperature loggers indicated that the water in the test tubes had reached 47 C, the rack was removed and pear disks of each isolate were quickly placed in their assigned tubes. We used 4 test tubes for each isolate (two disks per tube). This process took 5 minutes, during which water temperature in the tubes cooled considerably. Tubes were then returned to the water bath. When temperature reached 47 C in the tubes, timing of the heat treatment interval began.

After each time period elapsed, one test tube of each *Phytophthora* isolate was removed from the rack. The contents of each tube were poured into a sterile petri plate so that the disks would cool quickly. The disks were then used to inoculate surface-disinfested green D'Anjou pears as described under test 4, by using sterile forceps to insert the disk just below a flap of pear epidermis about 7-8 mm square. The epidermis was held down after inoculations were made by wrapping the pear with a 1.5 cm wide strip of parafilm. One pear was used for each time interval, so each pear had 8 inoculation spots (2 for each isolate). Pears were maintained at room temperature and monitored of the development of *Phytophthora* lesions as described under test 4. The test was conducted 5/1/2020.

Test 6 – Target 47 C for 0.5 to 2 h, *Phytophthora*-infected pear epidermal disks

This test used the same general methods as test 5. The depth of water in the water bath was increased to a depth of about 3 cm higher than the level of the water in the test tubes. We also added a third temperature datalogger that measured the water temperature in the water bath. The heating periods tested were 0.5. 1, 1.5, and 2 h. Disks were cut from pears that had been inoculated 3 days previously. Oogonia, but no oospores were seen in pear tissue for *P. cactorum* isolates. To ensure a quick drop in temperature at the end of the heating period, tubes were placed in cool (~20 C) water immediately upon removal from the water bath before further processing. This process was used in subsequent tests. We also included a control in this test that consisted of pear epidermal disks placed in water in a test tube kept at 21 C (70 F) for 2 h. Pear disks from all treatments and controls were used to inoculated pears as described for test 5. However, one of the duplicate pear disks from the 0.5 and 1 h treatments for all isolates was cut in half. One half disk was plated on PARP media and the other half was used to inoculate a pear. The test was conducted 5/11/2020.

Test 7 – Target 47 C for 20 to 40 minutes, *Phytophthora*-infected pear epidermal disks

Methods used were the same as in Tests 5 and 6 except that shorter heating periods of 20, 30, and 40 minutes were used. Disks were cut from pears that had been inoculated 7 days previously. Oospores were visible for *P. cactorum* isolates. As in test 6, the depth of water in the water bath was about 3 cm higher than the level of the water in the test tubes. We included a control in this test that consisted of pear disks placed in a test tube at 21 C (70 F) for 40 min. All pear pieces from all treatments and controls were used to inoculate pears as described for test 5. One of the duplicate pear disks from the two shortest heating periods (20 and 30 min in this test) were cut in half as in test 6, with one half being plated on PARP media and the other half being used to inoculate a pear. The test was conducted 5/15/2020.

Test 9 – Target 47 C for 20 to 40 minutes, pear epidermal and rhododendron leaf disks infected with *P. cambivora* or *P. kelmanii*

Note: there was no test 8 in this series.

Methods used in test 9 were the same as in tests 6 and 7 except that inoculated rhododendron leaves were also tested. Pear epidermal disks, from 13-day-old lesions for *P. kelmanii* and 21-day-old lesions for *P. cambivora*, were prepared as described above for previous experiments. To inoculate rhododendron, leaves were immersed in zoospore suspensions of each *Phytophthora* isolate for 3 days. Afterwards leaves were removed and placed in moist chambers for 14 days, by which time the leaves had become entirely brown. A sterile hole punch was used to cut 5 mm diameter disks from the discolored leaves. For both pear and rhododendron, three disks of each isolate were placed in each test tube, thus there were six disks per tube. Discs in water in control test tubes were incubated at 21 C (70 F) for 40 min. All pear discs and rhododendron leaf discs from all treatments and controls were plated on PARP media to assess viability after treatment. As in previous tests, PARP plates were observed for at least 2 weeks to detect growth of *Phytophthora*. The test was conducted 6/13/2020.

Test 12 – Target 47 C for 20 to 40 minutes, *P. cactorum* infected rhododendron leaf disks

This experiment followed the same procedures as test 9 except that only rhododendron leaf disks infected with two isolates of *P. cactorum* (CAC 3 and CAC 4, Table 5) were tested. For this test, disks were cut from rhododendron leaves 21 days after inoculation. The leaves inoculated with the CAC3 isolate were entirely dry and brittle by this point, whereas the leaves inoculated with the CAC4 isolate were still moist and pliable. No pear epidermal disks were used.

In the first run of this experiment (test 11) all but one of the disks floated to the surface of the water in the test tubes, presumably because of their low moisture content. Because we expected that they would not have been adequately heat-treated we discarded these disks and repeated the experiment. To prevent disks from floating in test 12, we placed wads of nylon tea bag fabric above the disks and pushed it below the water surface so the disks and the nylon fabric were completely submerged in the tubes. The test was conducted 6/25/2020.

3. Heat treatment of *Phytophthora*-infected plants

Tests 10 and 13

Q. lobata and *Q. agrifolia* seedlings grown in AB35 containers were individually inoculated with zoospore suspensions of *P. cactorum, P. cambivora,* or *P. kelmanii* and kept flooded for 3 hours after inoculation (Table 6). These plants were used for thermotherapy tests 18 to 38 days after inoculation. Prior to heat treatment, plants were watered daily but were not watered the day before the heat treatment. At 4-5 d, 9-10 d, and 15 d after inoculation, each individual plant was also baited with a green pear to assay for the presence of *Phytophthora* using the leachate protocol (http://phytosphere.com/BMPsnursery/test3_4bench.htm).

Table 6. Inoculated oaks in AB35 containers used for thermotherapy tests. QL=*Quercus lobata*; QA=*Quercus agrifolia;* CAC=*Phytophthora cactorum*; CAM=*P. cambivora*; KEL=*P. kelmanii*.

Host	Date host inoculated	*Phytophthora* species	Inoculation zoospores/plant	Date heat treated	Days inoculation to heat treatment
QL	18 May 2020	CAC3	3×10^5	6/14/2020	27
QA	18 May 2020	CAC4	2×10^5	6/25/2020	38
QL	19 May 2020	CAM	2×10^5	6/14/2020	26
QA	19 May 2020	CAM	2×10^5	6/25/2020	37
QL	27-May 2020	KEL	6×10^5	6/14/2020	18
QA	27-May 2020	KEL	6×10^5	6/25/2020	29

Heat treatments were conducted on 14 June 2020 for *Q. lobata* and 25 June 2020 for *Q. agrifolia*. Due to the involved nature of the heat treatment, the 30-minute heat treatment for each test plant was conducted and timed individually. Temperature probes recording temperature at 1-minute intervals were added to the center of each container prior to treatment so we could ensure that the coolest portion of the container reached the target temperature.

Each inoculated plant was held over a sink and irrigated with 50-51 C water until the temperature probe inserted into the rootball registered the target temperature of 47 C. A 30-minute countdown timer was started when 47 C was attained, but we continued to irrigate the plant with heated water until temperature reached at least 48 C to minimize temperature drop below the target in subsequent handling necessary before the plant could be transferred into the water bath. When the soil temperature reached 48 C, irrigation was stopped and the container was quickly placed in a plastic bag. The bag was wrapped tightly around the container and secured with a rubber band and the wrapped container was secured in the water bath.

Based on previous experience, the water bath thermostat was set at 48 C to compensate for the temperature fluctuation associated with thermostat cycling and slow temperature equilibration of the bagged containers. The water level in the bath was about 1-2 cm above the top of the container to minimize cooling of the soil surface, but water did not enter the container because the plastic bag extended well above the water line. After the countdown timer reached 30 minutes, each plant was removed from the water bath, the plastic bag was removed, and the soil temperature was quickly lowered by irrigating plants with cool water (~18 C) until the soil temperature reached 25 C or less.

Plants were irrigated daily after heat treatment. Plants were tested for *Phytophthora* presence 12, 22-28, and 43 days after heat treatment by baiting with green pears using the leachate procedure (http://phytosphere.com/BMPsnursery/test3_4bench.htm) on each individual container. On 15 September, 82 (*Q. agrifolia*) to 93 days (*Q. lobata*) after heat treatment, root systems were examined and compared to noninoculated, non heat-treated plants. The entire root system of each plant was placed in a plastic bag, flooded, and baited with a green pear. Pears were examined for symptoms beginning after 3 days of incubation. Pears were removed from bags after 5 days and continued to be evaluated for symptoms for at least an additional 3 days (8 days from the start of baiting).

Results

Plant thermotolerance experiments

Test 1 – Target 45 C for 34 h

Our initial setup could not maintain temperature at our original target of 50 C, so we reduced the target to 45 C. We were able to maintain rootzone temperatures at about 45 C in the smaller AB35 containers at both the outer wall and container center (Figure 3). It took several hours for these containers to reach 45 C.

Measured temperatures in the center of the larger AB46 containers were about 3 C lower than in the AB35s and never attained the target temperature (Figure 3). Excluding the initial heat-up period, the average of all readings over 40 C for the center of the AB46s was 41.7 C. We assume that evaporative cooling from the soil surface in the containers kept the center of the soil mass from equilibrating at the same temperature as the outer edge adjacent to the container wall. In addition, measurements taken over the heat cycle with a digital thermometer (Thermoworks Thermopop) showed that the top 1 cm of the soil was several degrees cooler than soil at 6 cm depth. These cooler temperatures may account for the lack of damage seen in these plants.

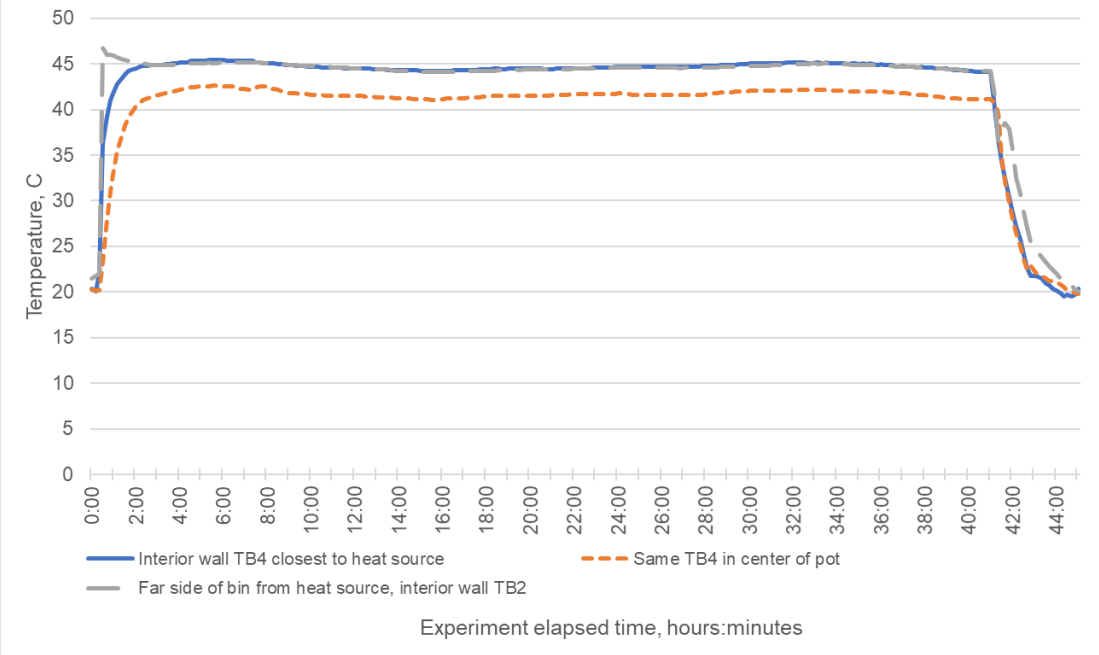

Figure 3. Test 1. Potting media temperatures measured in containers held in a water bath maintained at 45 C (113 F) for more than 34 hours. Hot water at 52 C was added to bath starting at 30 min elapsed time. Water bath temperature could not attain initial target of 50 C, so the target was reduced to 45 C. Blue line – probe at edge of the AB46 closest to heat source, dashed orange line – probe placed at center of same AB46, gray dashed line – probe placed at edge of an AB35 furthest from heat source.

Evaluation of the plants two months after treatment showed markedly different responses to heat treatment. *Cornus sericea* (AB35), *Symphoricarpus albus* (AB35), *Ribes sanguineum* (AB46), and *Rubus parviflorus* (AB46), were in acceptable condition, whereas *Juncus xiphioides, Lonicera hispida,* and *Rubus ursinus* (all in AB35 containers) were severely damaged or killed by the heat treatment. When evaluated 4/20/20 (84 days from start of treatment, Table 7), the condition of *Cornus sericea, Symphoricarpus albus, Ribes sanguineum*, and *Rubus parviflorus* were still acceptable and plants were pushing new growth. These plants continued to survive through the summer of 2020.

Table 7. Plant condition at time of treatment and on 20 April 2020, 77 -84 days after heat treatment as described for Tests 1, 2, and 3. Brown shading indicates unacceptable plant condition, green shading indicates plant condition acceptable.

			Number of containers – flooding and damage / days post treatment		
Species	**Container size**	**Phenology / condition at treatment**	**Test 1: target 45 C – 34 h / 84 d**	**Test 2: target-41 C – 96 h / 82 d**	**Test 3: target 50 C – 1.5 h / 77 d**
Cornus sericea	AB35	Dormant / moderately large plants (~8 mm basal diam)	2 – acceptable, growing	2- live but unacceptable	
Juncus xiphioides	AB35	Vegetative, no active growth / plants a bit senescent with many dead and chlorotic leaves, underpotted	3 – live but unacceptable	3 (2 flooded) – all unacceptable	3 unflooded – virtually dead / 3 flooded all dead (exceeded time +temp target)
Lonicera hispida	AB35	Vegetative, no active growth / very small plants with purple coloration in most leaves	3 – 1 dead, others unacceptable shoot dieback	3 – all dead, 1 flooded, it died first	
Rubus ursinus	AB35	Vegetative, no active growth / very small nonwoody plants with 2-3 leaves	3 – all dead	1 flooded – dead, 2 not flooded acceptable	
Symphoricarpus albus	AB35	Dormant / small but variable size (≤ 5 mm basal diam)	3 – acceptable, growing	3 (2 flooded) – all live acceptable	
Ribes sanguineum	AB46	Breaking dormancy, pushing new unexpanded leaves up to 1 cm / large plants (~10 mm basal diam), underpotted	2 – acceptable	1 flooded – unacceptable, 1-nonflooded acceptable	2 flooded – unacceptable, dead and near dead (below time target)
Rubus parviflorus	AB46	Dormant / large plants (~10 mm basal diam)	2 – growing, acceptable	1 flooded – dead, 1 nonflooded – acceptable	2 flooded – acceptable (below time target)

Test 2 – Target 41 C for 96 h

In test 2, soil temperatures at the AB35 and AB46 container edges were around 41 C. The probe in the center of a AB35 recorded temperatures that were nearly identical to those measured at the interior wall the other monitored AB35 container (solid lines, Figure 4). Slight fluctuations due to the cycling of the heating element registered on the thermocouple probes, resulting in the sawtooth pattern seen in the temperature plot (Figure 4). The probe in the center of the monitored

AB46 container did not register these variations in temperature (gray dashed line in Figure 4), indicating the temperature of the larger soil mass was less responsive to these temperature variations.

As in test 1, the temperature in the center of the AB46 container (gray dashed line in Figure 4) was several degrees lower than target for about the first 44 hours. At that time, we added additional heated water to the water bath to raise the water level about 1.5 cm above the soil surface in the containers, which were kept from flooding by the plastic bags. However, the monitored AB46 was one of 7 containers in this test that flooded due to leaks that developed in the plastic bags. The flooding of the AB46 container raised its temperature to about 41 C (the target temperature). Due to evaporation, water level in the bath dropped and we added more water at about 70 hours into the test. As seen in Figure 4 (gray dashed line), the temperature at the center of the AB46 was trending lower as the water level dropped and rose back to 41 C when additional water was added at the 70-hour mark. The temperature again began to drop near the end of the test due to falling water levels. These observations suggested that heat transfer to the center of larger containers was more successful if the soil in the container was saturated and confirmed our suspicion that evaporative cooling from the soil surface exerted a significant cooling effect.

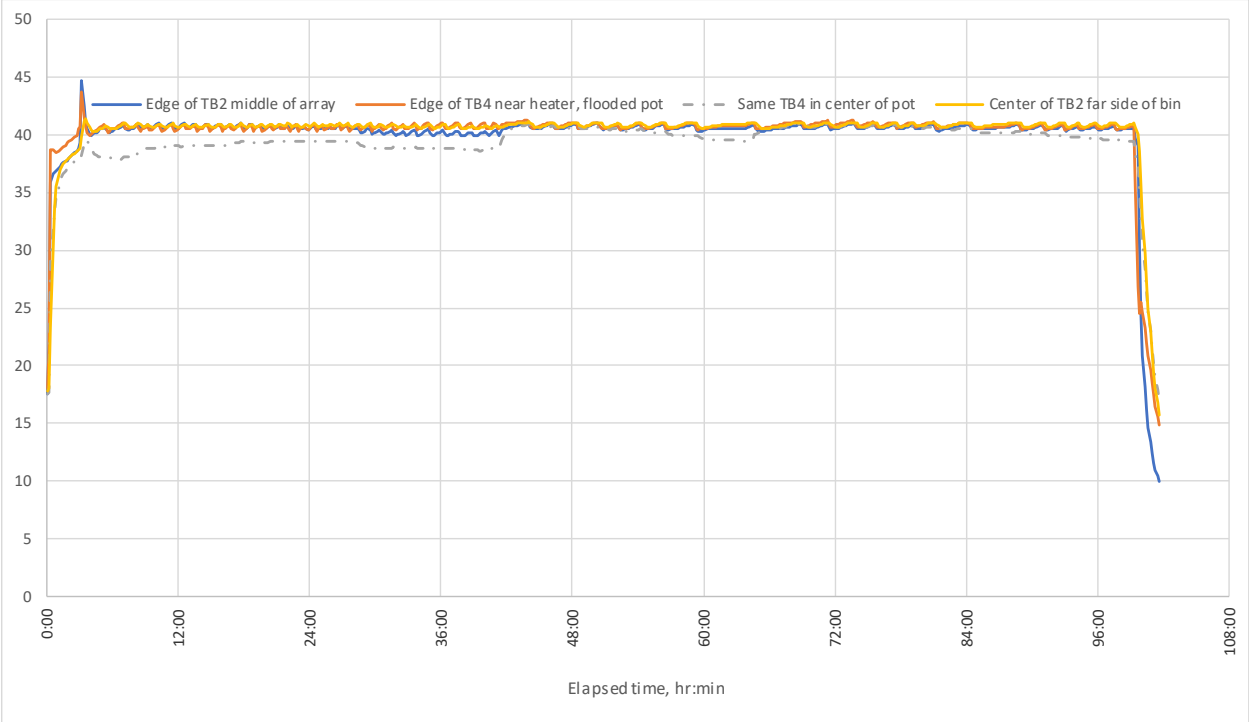

Figure 4. Test 2. Temperatures measured in containers held in a water bath maintained at 41 C (113 F) for 96 hours. Blue line – probe at edge of AB35 in center of pot array, orange line – probe at edge of a AB46 near the heater, gray dashed line – probe placed in center of same AB46, yellow line – probe paced in center of AB35 on far side of water bath from heater.

Only *Symphoricarpus albus* tolerated this heat treatment with little or no adverse effects. The other species tested were either severely damaged, or in some cases, only flooded plants were damaged (Table 7).

Test 3 – Target 50 C for 1.5 h

The 1000 W bucket heater used in this test was easily able to maintain the water bath at the higher target temperature. We initially placed the thermostat sensor in the center of an AB46 container, instead of against the container edge as we had done for tests 1 and 2. Due to the time lag associated with heat transfer to the center of the pot, this sensor placement led to overshooting the target temperature in the smaller AB35 containers by several degrees C (Figure 5). When we noticed this, we moved the temperature sensor to the interior edge of a pot, which corrected the problem (2.25 h mark in Figure 5).

Plants had been held outside overnight prior to the morning start of this test and initial soil temperatures were between 9 and 13 C (Figure 4). The target temperature of 50 C was not attained in the center of the monitored AB35 until 70 minutes into the test and the AB46s did not attain the target temperature until more than 2 hours into the test (Figure 5). Because it was not possible to remove the AB35 plants without risking capsizing the AB46 plants, the AB35 plants ended up with significantly longer exposure to elevated temperature.

Three of the AB35s flooded within 15 minutes of the study start. The AB46 with the center temperature probe (dashed gray line Figure 5) flooded about 80 minutes after the test was started. At that point, we flooded the 3 remaining AB46s with 50 C water. The 3 unflooded AB35s remained unflooded. The soil surface temperature measured in one AB46 remained below 50 C, even though the water level in the water bath was about 1.5 cm above the level of the water in the flooded container. Note that the plastic bag still separated the water bath from the water within the bag. Plants were removed from the water bath after 3 hours and 5 minutes. Over the length of the test the AB35 plants reached higher maximum temperatures (52.8 C) and were held at 50 C or above for 100 minutes (Figure 5). These plants were killed by the heat treatment (Table 7). In comparison, the AB46 plants did not exceed 50.7 C and were at 50 C or above (at least in the center of the pots) for 60 minutes. The *Ribes sanguineum* was killed by the treatment, but the *Rubus parviflorus* was not (Table 7).

From this test it was evident that all containers being treated needed to be of the same size to achieve uniform heating. In addition, temperature remained elevated for an extended period after pots were removed from the water bath, suggesting that special efforts would be needed to cool plants rapidly after heat treatment.

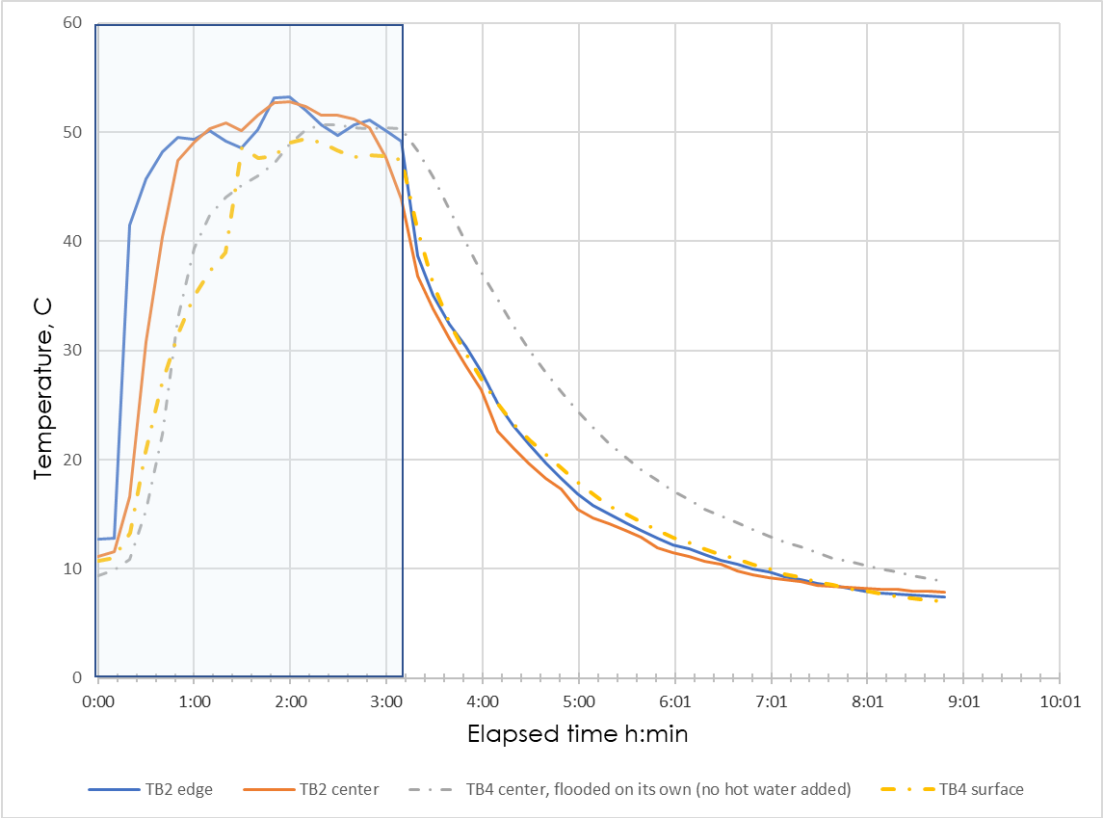

Figure 5. Test 3. Temperatures measured in pots held in a water bath maintained at 50 C (113 F) for 3 hours. Blue box represents time in water bath. Solid lines = AB35 containers: Blue line – probe placed against edge of the AB35 furthest from the heater in the pot array, orange line – probe placed in center of adjacent AB35 also far from heater. Dashed lines= AB46 containers near the heater: Gray dashed line – probe placed in center of AB46, yellow dashed line – probe paced at surface (1 cm depth) of adjacent AB46.

Test 4A – Target 47 C for 4 h

Based on the damage associated with the 50 C test we decided to try a slightly cooler temperature that should be capable of killing *Phytophthora* in a fairly short time. In this test, we started by partially submerging each pot in a plastic bag in a 47 C water bath. We added additional 47 C water to each pot and then fully submerged pots into the 47 C water bath. We thought this method would attain more uniform and rapid temperature equilibration, but flooding each pot with 47 C water only raised the soil in the pots to 30-35 C (Figure 6). We moved the thermostat for the bucket heater from the water into one of the pots, but this caused the water in the bath to overshoot the desired treatment temperature by several degrees (Figure 6). At that point we turned off the bucket heater to allow the pots to equilibrate to the water bath temperature without further overshooting the desired temperature. When temperature in the pots reached 47 C we restarted the bucket heater. Containers with only *Phytophthora* pear epidermis disks in potting media (dotted lines) heated much more quickly than containers with plants (Figure 6). This is likely due to both the different thermal properties of the root mass and the greater level of compaction (i.e., reduced porosity) of the containers with plants compared to those with only potting soil and inoculum packets.

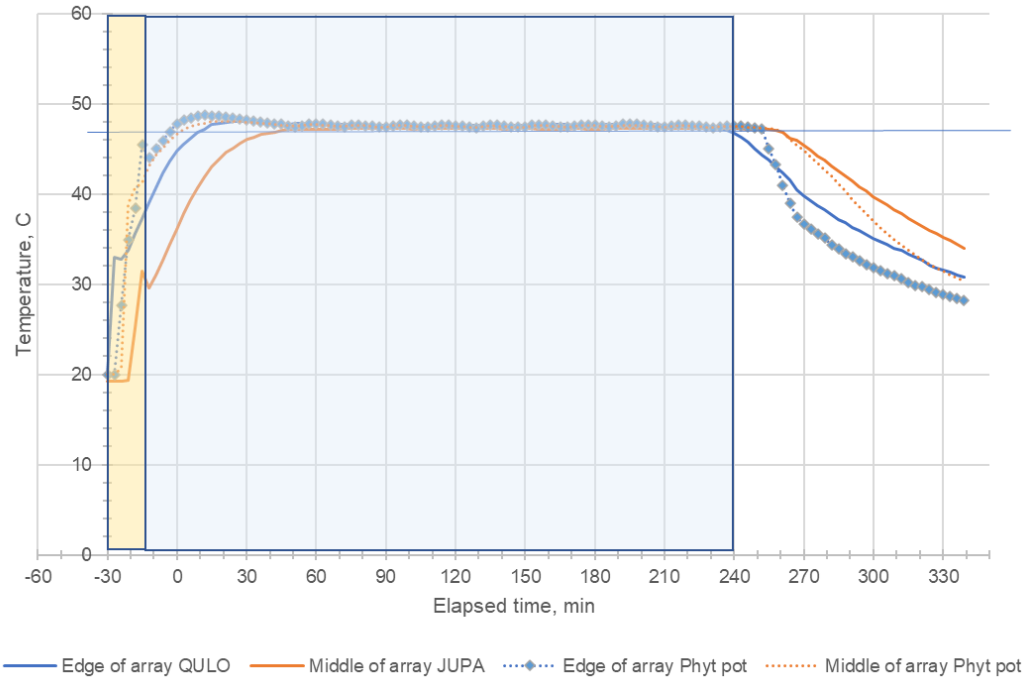

Figure 6. Tests 4A and B: Temperatures measured in AB35 containers in plastic bags containing either plants or *Phytophthora* inoculum in potting media treated in a water bath at 47 C (117 F, horizontal blue line) for 4 hours. Data loggers recorded temperature of soil in the center of four of the pots at 3-minute intervals. Initial temperature of soil in pots was about 20 C and temperature increased as 47 C water was added to pots (yellow area). Pots were then fully submerged in the water bath (blue area). Time of treatment was started when the first pot reached 47 C (elapsed time = 0 min). Temperature registered 46.9 C at -3 minutes. The temperature of the water bath was raised above 47 C for about 50 minutes to get all containers up to the desired treatment temperature. Containers were removed from water bath after probes showed most had been at 47 C for four hours. Pots were unbagged and allowed to drain.

All containers were removed from the water bath at the same time as the *Phytophthora* containers, but unbagging and draining each container took time, as reflected by the different rates of cooling seen in Figure 5. Due to lags associated with reaching the target temperature and subsequent cooling, some plants were exposed to the 47 C temperature treatment for times that were below or above the 4 h (i.e., 240 min) target time (range 213 to 255 min for the 2 temperature probes plotted in Figure 6).

Some heat damage symptoms were visible on leaves of *Q. lobata* and *J. xiphioides* on the day after the heat treatment, mainly affecting leaves that were in contact with the submerged portion of the plastic bags around the containers. However, by 8 days after treatment, all the heat-treated plants except *J. patens* showed unacceptably high levels of damage (Table 8). Only the *J. patens* and one *Q. lobata* were still alive when evaluated about 6 months after treatment (Figure 6). The temperature rise in the instrumented *J. patens* lagged behind the other monitored plants, resulting in the shortest heat treatment (213 min exposure time at 47 C). These plants had very high root density (Figure 6, bottom) and it is possible that this may have resulted in slower heating. The position of the pot (in the center of the array versus edge for *Q. lobata*) is probably not related to the observed lag in heating, given that the 2 monitored containers with *Phytophthora* packets at the edge and center positions in the container array heated at similar rates (dotted lines Figure 6).

Table 8. Test 4A. Condition of plants 8 days after heat treatment at 47 C for 3.5-4 hours. Two replicate flooded containers for each plant species.

Species	**Container size**	**Phenology / condition after heat treatment of 47 C for 3.5-4 hours**
Juncus patens	AB35	Acceptable, no noticeable change in condition, but not growing
Juncus xiphioides	AB35	100% leaf scorch – both plants
Quercus agrifolia	AB35	100% leaf scorch 1 plant, 2nd plant – all leaves scorched, 30% of leaves still with green midribs.
Quercus lobata	AB35	100% leaf scorch 1 plant, 2nd plant – all leaves scorched, most leaves with green island around midribs.
Ribes malvaceum	AB35	100% leaf scorch – both plants

Figure 7. Test 4A. Appearance on 4/30/20 (top) and 10/14/20 (center) of plants treated at 47 C for about 4 h on 4/22/20. Species from left to right are *Ribes malvaceum*, *Quercus agrifolia*, *Q. lobata*, *Juncus xiphioides*, and *J. patens*. Bottom image shows root systems of *J. patens* (top) and *Q. lobata* (bottom) plants used in Test 4 before heat treatment. The greater root mass of the *J. patens* may in part explain the slower increase in temperature seen in this species compared to *Q. lobata* (see Figure 6).

Conclusions

Several results were obvious from these trials.

1. Raising temperatures of soil in containers to target temperature takes longer as the size of the pot increases. Therefore, containers of different sizes need to be treated separately in order to keep heating uniform.

2. Even with the relatively small containers used in these trials, it was more difficult to maintain uniform temperatures as container size increased. This was related to both the slow transfer of heat from the outside to the inside of the soil volume in the container and the greater surface area of the container top, which allows for greater radiative heat loss and evaporative cooling.

3. Due to these heat loss processes, the top of the container needs to be submerged under hot water to ensure temperatures reach target throughout the entire soil profile of the pot. However, this also exposes the base of the stem and low leaves to the elevated temperatures, which may increase plant damage.

4. Because of the slow rate of heating from an external source such as a water bath, better control of the temperature treatment can be managed by ensuring that container soils are already warm before treatment. This can be best be achieved by pouring warmed water though the container until it approaches or attains the target temperature, but even this process will be faster if the containers have been held in a warm location in advance.

5. From a practical standpoint, long heat treatment times (longer than could be completed in a single work day) were very cumbersome, difficult to control, and did not offer enough of a plant survival advantage to be worth the level of effort required.

6. Temperature probes need to be placed into the heated units (containers in these tests) to monitor the temperature attained at the target site during the treatment. The rate of heat transfer from an external source, such as a water bath or heated chamber, to the center of the rootball in a container plant is slow (70 to 120 minutes in our most efficient test, test 3). The exposure time and final temperature reached in the roots and soil of a container plant target cannot be determined by only monitoring how long a container plant was in a water bath or chamber of given temperature.

Based on these experiments, plant tolerance of a 50 C treatment was not encouraging, so we focused on a slightly lower temperature (47 C) that had the potential to cause less plant damage, particularly if the exposure period could be kept short enough. Though the 47 C × 4 h heating regime was not well tolerated in test 4A, both the maximum temperature and total treatment time were exceeded in some of the test plants (Figure 6) and the plants with the lowest temperature duration (*J. patens*, 213 minutes) tolerated the treatment. Based on the literature review (Table 1), 4 h at 47 C could kill *Phytophthora*, but the minimum exposure time required at this temperature was not established. We therefore initiated a series of tests to determine the minimum treatment time at 47 C needed to kill *Phytophthora* inoculum.

2. *Phytophthora* heat sensitivity tests

Test 4B – *Phytophthora* survival in potting media at 47 C for 4 h (4/22/2020)

As noted above, this test was conducted concurrently with test 4A. No *Phytophthora* was recovered from any of the heat-treated *Phytophthora* pear disks assayed by either plating on agar or inoculating green pears (Table 9). In contrast, all four of the *Phytophthora* isolates used were recovered from non-heated control disks using both agar plates and inoculated pears. This confirmed that 4 h at 47 C was sufficient to kill the isolates of the three common nursery *Phytophthora* species tested.

Table 9. Survival of *Phytophthora* species in infected pear disks after treatment in 47 C water for various lengths of time from tests 4B, 5, and 6. – no recovery, + *Phytophthora* recovered.

Test	Treatment duration	*P. kelmanii*	*P. cambivora*	*P. cactorum 3*	*P. cactorum 4*
Test 4B	4 h	–	–	–	–
	Nonheated control (0 h)	+	+	+	+
Test 5	3.5 h	–	–	–	–
	3 h	–	–	–	–
	2.5h	–	–	–	–
	2 h	–	–	–	–
Test 6	2 h	–	–	–	–
	1.5 h	–	–	–	–
	1.0 h	–	–	–	–
	0.5 h	–	–	–	–
	Nonheated control (0 h)	+	+	+	+

Test 5 – Target 47 C for 2 to 3.5 h, *Phytophthora*-infected pear epidermal disks

Based on our experience with the concurrent tests 4A (plant thermotolerance) and 4B (*Phytophthora* thermotolerance in potting media), we determined that it would be easier to first establish the minimum treatment time at 47 C needed to kill *Phytophthora* and subsequently test plant tolerance of this regime. Because it was difficult to precisely control the timing of heat exposures in soil-filled containers, we used test tubes filled with preheated water to expose disks cut from *Phytophthora* lesions on pears to 47 C. In this test, we tested a series of exposures times less than 4 h: 3.5, 3, 2.5, and 2 h.

In this test, the temperature of water in the test tubes dropped markedly when they were removed from the water bath to add the *Phytophthora*-infected pear epidermal disks (Figure 8). After being placed back in the water bath, about 25 minutes elapsed before the water in the test tubes reached 47 C again. Timing of the heat-treatment periods began when temperature in the tubes reached 47 C (elapsed time = 0 min in Figure 8). Cycling of the thermostatically-controlled heater resulted in regular oscillation in temperature during the treatment period (Figure 8).

No viable *Phytophthora* was detected from any of the heat-treated disks when assayed by pear lesions (Table 9). This indicated that the lethal exposure time for *Phytophthora* was no more than 2 hours at 47 C. The time lag before the first 47 C reading was attained added some uncertainty to this estimate because water temperatures fell just short of this temperature (46.7-46.9 C) for about 12 minutes before attaining 0 C where the 0 minute mark was recorded (Figure 8).

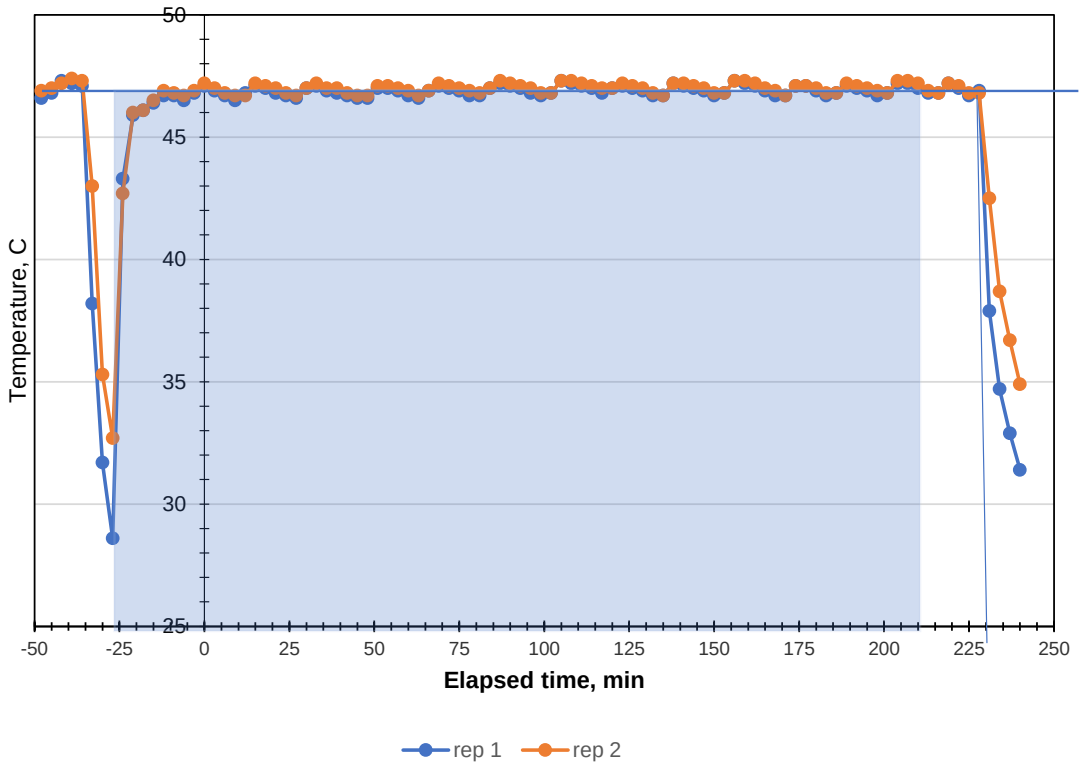

Figure 8. Test 5. Temperatures in two duplicate test tubes (rep 1 and 2) with temperature probes recording at 3-minute intervals in a 47 C water bath. After the test tubes were preheated to 47 C, the test tube rack was removed from the water bath and pear disks were quickly added to test tubes (temperature drop at about -35 to -25 minutes). Tubes were returned to the water bath after infected disks were added (start of blue shaded area). Water in tubes reheated to the 47 C target in about 25 minutes. Test tubes containing pear disks were removed from the water bath 2, 2.5, 3, and 3.5 h (end of blue shaded area) after reaching 47 C (elapsed time = 0). Horizontal blue line= 47 C. Test tubes containing data loggers were removed from water bath at 228 minutes elapsed time.

Test 6 – Target 47 C for 0.5 to 2 h, *Phytophthora*-infected pear epidermal disks

Because all heat treatment times used in test 5 killed *Phytophthora* inoculum, in test 6 we lowered exposure times to 2 h and less: 2, 1.5, 1, and 0.5 h. Other parameters were kept the same except that a nonheated control was added. An additional datalogger that recorded water temperature in the water bath was also added.

As seen in test 5 (Figure 8), temperatures in the preheated test tubes dropped to between 28 and 33 C when the test tubes were removed from the water bath and *Phytophthora*-infected pear disks were added (Figure 9). However, the tubes reheated to the 47 C target temperature in about 7 minutes after they were returned to the water bath, rather than the 25 minutes observed in test 5. This difference in the rate of reheating was apparently due to the fact that we increased the water depth in the bath above the water level in the tubes, from 0.5 cm in test 5 to about 3 cm in test 6. When returned to the water bath, temperatures in the tubes increased steadily and reached 47 C in one thermostat cycle (Figure 9) avoiding the 12-minute near miss of the target temperature that occurred in test 5 (Figure 8). Starting with this test, tubes removed at the end of each treatment

interval were immediately placed in cool water so that all tubes would begin to cool simultaneously. In figure 9, the tube with the rep 2 (orange) temperature probe was removed from water bath at same time as test tubes receiving the 2 h treatment. Cooling began immediately upon removal and the temperature in this tube dropped to 25 C within 2 minutes.

No viable *Phytophthora* was detected from any of the heat-treated disks when assayed by pear lesions (Table 9). In addition, no *Phytophthora* mycelium grew from the half pear disks from the 0.5 and 1 h heat treatments that were plated on PARP. In contrast, lesions formed within 2 days around control pear disk inoculation spots. This indicated that the lethal exposure time for *Phytophthora* was no more than 0.5 hours at 47 C, well below the value expected from published literature.

One disk in the 30-minute heat treatment tube had adhered to the wall of the tube above the water line but was not noticed until the end of the treatment. Due to evaporative cooling, this disk would not have received a 47 C × 30 minute heat treatment and this disk caused a *Phytophthora* lesion when inoculated into a green pear. Results for this disk are not included in Table 9 because it was not subjected to the target treatment. However, this result illustrates how small details in the experimental system could give rise to inaccurate results.

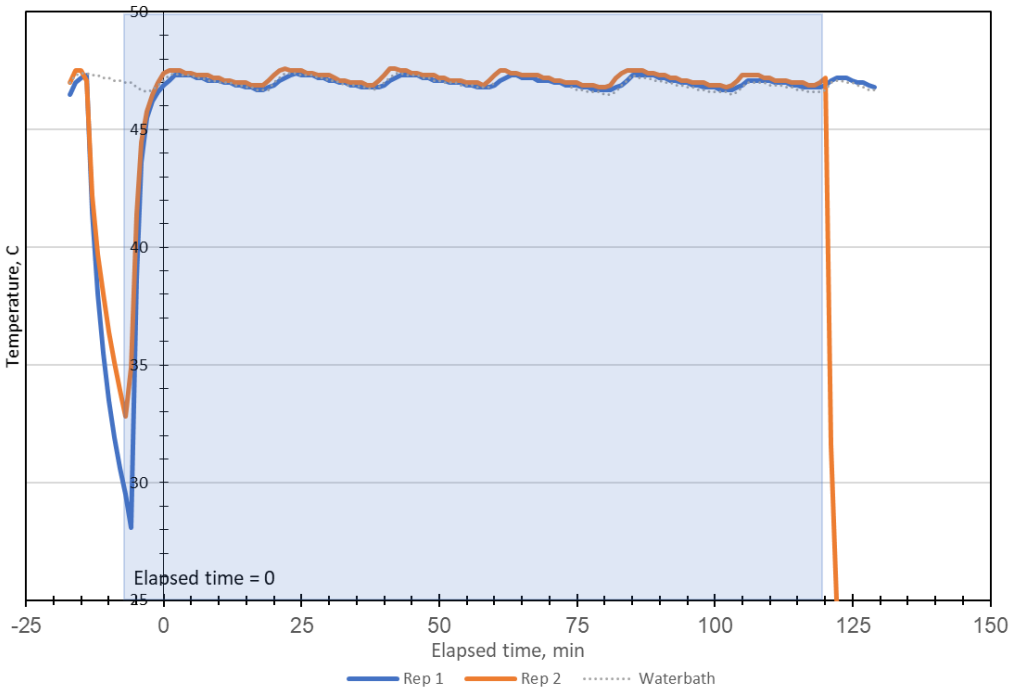

Figure 9. Test 6. Temperatures in two duplicate test tubes (rep 1 and 2) with temperature probes recording at 1-minute intervals in a 47 C water bath. Dotted gray line shows temperature readings for the water bath. After the test tubes were preheated to 47 C, the test tube rack was removed from the water bath and pear disks were quickly added to test tubes (temperature drop at about -15 to -7 minutes). Tubes were returned to the water bath after infected disks were added (start of blue shaded area). Water in tubes reheated to the 47 C target in about 7 minutes. Test tubes containing pear disks were removed from the water bath 0.5, 1, 1.5, and 2 h (end of blue shaded area) after reaching 47 C (elapsed time = 0). The tube with the rep 2 temperature probe (orange line) was removed from water bath and placed in cool water at same time as test tubes receiving the 2 h treatment.

Test 7 – Target 47 C for 20 to 40 minutes, *Phytophthora*-infected pear epidermal disks

Data from test 6 indicated that the four *Phytophthora* isolates we tested were killed by a 30-minute exposure to 47 C. In tests 7, 9 and 11, we focused on confirming this unexpected finding by testing exposure times of 20, 30, and 40 minutes. Test 7 was conducted following the same procedures as test 6, except for the use of these shorter heat treatment intervals.

In test 7, we saw the same strong temperature decline when the preheated test tubes were removed from the water bath (Figure 10) that was seen in tests 5 and 6 (Figures 8 and 9). Water in the tubes reheated to 47 C within 4 to 6 minutes of being returned to the water bath (Figure 10). The speed with which the water in the tubes reheated is comparable to test 6, which used the same water level in the water bath (about 3 cm above test tube water level). Timing of the heat treatment periods began when temperature in both tubes reached 47 C (elapsed time = 0 min in Figure 10).

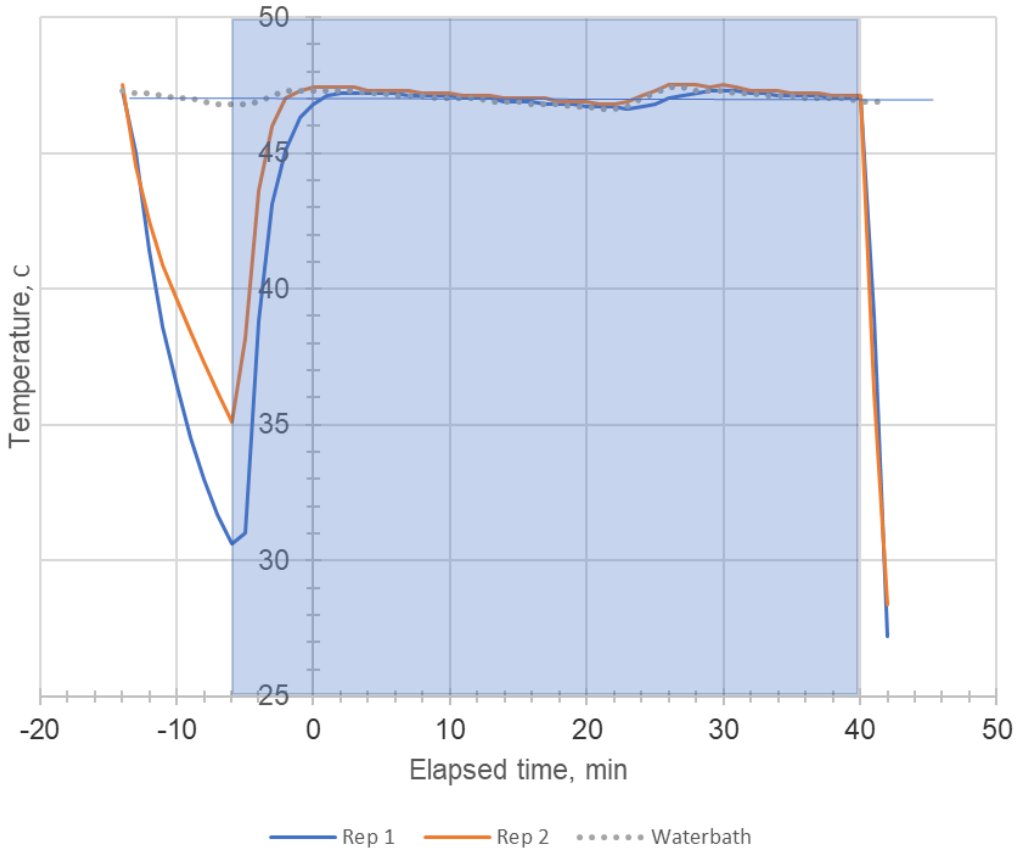

Figure 10. Test 7. Temperatures in two duplicate test tubes (rep 1 and 2) with temperature probes recording at 1-minute intervals in a 47 C water bath. Dotted gray line shows temperature readings for the water bath. After the test tubes were preheated to 47 C, the test tube rack was removed from the water bath and pear disks were quickly added to test tubes (temperature drop at about -15 to -6 minutes). Tubes were returned to the water bath after infected disks were added (start of blue shaded area). Water in tubes reheated to the 47 C target in 4 to 6 minutes. Test tubes containing pear disks were removed from the water bath 20, 30, and 40 minutes after reaching 47 C (elapsed time = 0). Both tubes with temperature probes were removed from water bath at same time as test tubes receiving the 40-minute treatment.

The lesions for the pear disk inoculum in this test were 7 days old and the *P. cactorum* disks contained oospores. As in the previous tests, no viable *Phytophthora* was detected from any of the heat-treated disks when assayed by pear lesions or by isolation on PARP (Table 10). In contrast, *Phytophthora* lesions formed within 2 days around areas where pears were inoculated using nonheated control disks. In this test, even the shortest heat treatment of 20 minutes at 47 C was effective.

Table 10. Survival of *Phytophthora* species in infected pear disks (tests 7 and 9) and rhododendron (rhod) leaf disks (tests 9 and 12) after treatment in 47 C water for 20 to 40 minutes. – no recovery, + Phytophthora recovered. Note that the results for P. cactorum CAC3 in test 12 are noninformative because the nonheated controls were not viable.

Treatment, temperature duration	*P. kelmanii*	*P. cambivora*	*P. cactorum* CAC3 (from toyon)	*P. cactorum* CAC4 (from coffeeberry)
Test 7 pear disks				
40 min	–	–	–	–
30 min	–	–	–	–
20 min	–	–	–	–
Nonheated control (0 min)	+	+	+	+
Test 9 pear / rhod leaf disks				
40 min	–/–	–/–		
30 min	–/–	–/–		
20 min	–/–	–/–		
Nonheated control (0 min)	+/+	+/+		
Test 12 rhod leaf disks				
40 min			–	–
30 min			–	–
20 min			–	–
Nonheated control (0 min)			–	+ (2 of 3)

Test 9 – Target 47 C for 20 to 40 minutes, *P. cambivora* and *P. kelmanii* infected pear epidermal and rhododendron leaf disks

In tests 9 and 12, we tested inoculum produced in inoculated rhododendron leaves to see whether it exhibited different temperature sensitivity than infected pear epidermal disks. Test 9 included both infected pear epidermal disks and rhododendron leaf disks for two *Phytophthora* species, *P. cambivora* and *P. kelmanii*. Disks were cut from rhododendron leaves 14 days after inoculation. Temperature graphs for test 9 (Figure 11) were similar to those of tests 6 and 7. Tubes reheated to 47 C about 6 minutes after being replaced in the water bath.

Phytophthora mycelium was observed growing from all the control pear epidermal and rhododendron leaf disks one day after being plated on PARP (Table 10). However, no *Phytophthora* was detected from any of the heat-treated pear or rhododendron disks, which was consistent with the results from test 7 (Table 10).

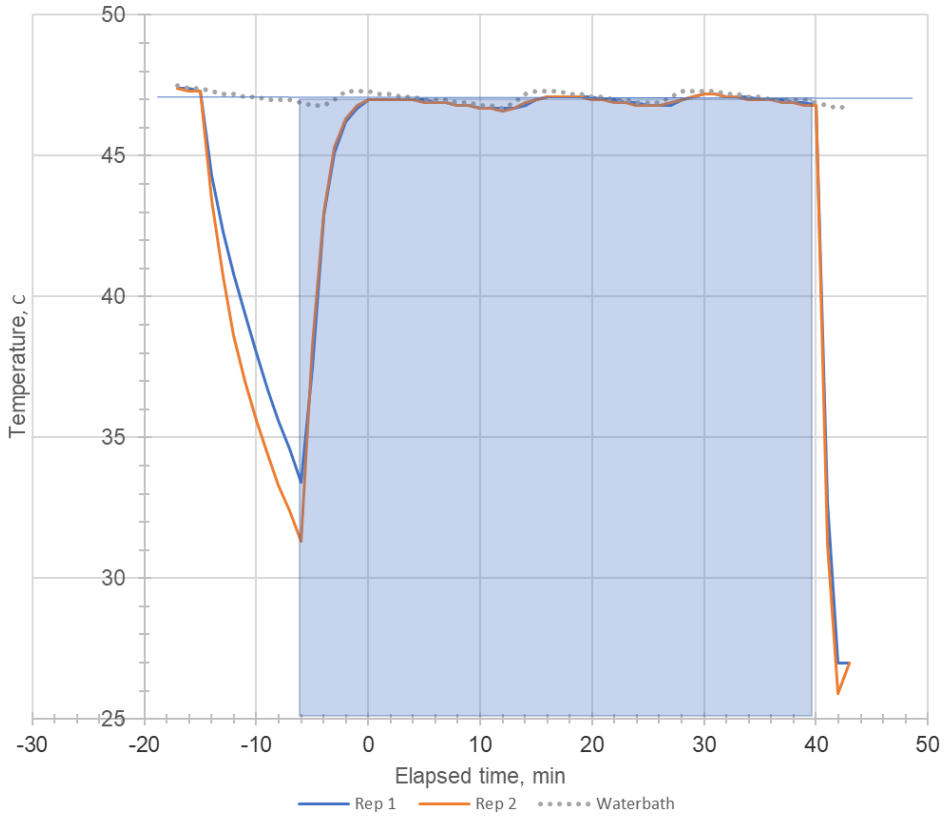

Figure 11. Test 9. Temperatures in two duplicate test tubes (rep 1 and 2) with temperature probes recording at 1-minute intervals in a 47 C water bath. Dotted gray line shows temperature readings for the water bath. After the test tubes were preheated to 47 C, the test tube rack was removed from the water bath and pear and rhododendron leaf disks were quickly added to test tubes (temperature drop at about -16 to -6 minutes). Tubes were returned to the water bath after infected disks were added (start of blue shaded area). Water in tubes reheated to the 47 C target in about 6 minutes. Test tubes containing pear and rhododendron leaf disks were removed from the bath 20, 30, and 40 minutes after reaching 47 C (elapsed time = 0). Both tubes with temperature probes were removed from water bath at same time as test tubes receiving the 40-minute treatment.

Test 12 – Target 47 C for 20 to 40 minutes, *P. cactorum* infected rhododendron leaf disks

In this test, rhododendron leaf disks infected with the two *P. cactorum* isolates were tested. No pear epidermal disks were used.

Temperature graphs for test 12 (Figure 12) were similar to those of tests 6, 7, and 9. Tubes reheated to 47 C after about 6 minutes in the water bath. Two of the three nonheated control rhododendron disks inoculated with CAC4 produced *Phytophthora* mycelium on PARP, whereas no *Phytophthora* was detected from any of the heat-treated disks (Table 10). As noted above, the leaves used for the CAC3 disks were dry and brittle at the time the experiment. No *Phytophthora* mycelium grew from any of the nonheated control leaf disks from isolate CAC3 (Table 10), so

the results for the test with this isolate were noninformative. This experiment did not assay viability by inoculation into pears.

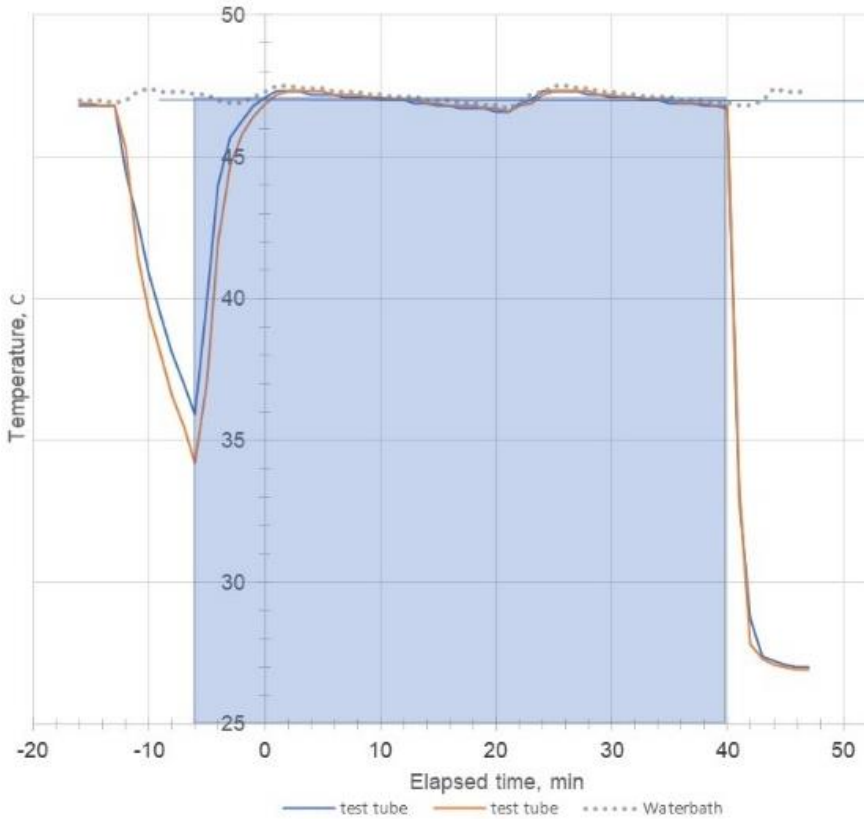

Figure 12. Test 12. Temperatures in two duplicate test tubes (rep 1 and 2) with temperature probes recording at 1-minute intervals in a 47 C water bath. Dotted gray line shows temperature readings for the water bath. After the test tubes were preheated to 47 C, the test tube rack was removed from the water bath and pear and rhododendron leaf disks were quickly added to test tubes (temperature drop at about -14 to -6 minutes). Tubes were returned to the water bath after infected disks were added (start of blue shaded area). Water in tubes reheated to the 47 C target in about 6 minutes. Test tubes containing rhododendron leaf disks were removed from the bath 20, 30, and 40 minutes after reaching 47 C (elapsed time = 0). Both tubes with temperature probes were removed from water bath at same time as test tubes receiving the 40 minute treatment.

Conclusions

Results from this series of *Phytophthora* heat sensitivity tests were quite consistent and showed that all three *Phytophthora* species tested (4 isolates total) were nonviable after exposure to 47 C in water for 20 minutes or longer. These results were obtained in two types of infected plant tissues; infected pear epidermis and infected rhododendron leaves. This suggests that the high level of temperature sensitivity was not uniquely related to the inoculum source. It is also relevant to our study that the heat-treated inoculum was produced and present in host tissue, since this is the situation that needs to be addressed in thermotherapy of live plants.

P. cactorum is homothallic and single isolates produce numerous resistant oospores. Mature oospores were observed in multiple tests, including test 7, in which both *P. cactorum* isolates

failed to grow after 20-minute exposures to 47 C. Both *P. cambivora* and *P. kelmanii* are heterothallic, although we have occasionally observed oospores in cultures of *P. cambivora*. We could find no references suggesting that either isolate produces asexual resistant spores such as chlamydospores. None of the *Phytophthora* species we tested are considered to be especially tolerant of high temperatures. More heat-tolerant *Phytophthora* species might show greater tolerance to exposure to 47 C.

3. Heat treatment of *Phytophthora*-infected *Q. lobata* and *Q. agrifolia* seedlings

Based on the studies reported in section 2 above, we selected 47 C × 30 minutes as a test thermotherapy regime for treating live plants. This temperature × time regime was shown to be effective against the *Phytophthora* species used in several tests. It was also longer than the effective 20-minute treatment, which allowed for some margin of error. Treating container-grown plants in a water bath is a more complicated situation than treating leaf disks, so we wanted to allow for some buffer beyond the minimum effective time.

We had not previously tested plants for their ability to tolerate a 30 minute treatment at 47 C. Our earlier test of 47 C for 4 h (test 4A) resulted in unacceptable plant damage (Table 8). However, in test 1 some of the tested plants tolerated 45 C for 34 h and in test 3, some plants survived an hour at 50 C. Hence, it seemed possible that 47 C × 30 minutes could be tolerable for the test plants.

In these tests, temperature probes were placed at the center of the rootball of each plant and heat treatments were applied individually to ensure that they were as close to the target regime as possible.

Test 10 – *Phytophthora*-infected *Quercus lobata* seedlings treated at 47 C for 30 minutes

Temperature graphs for three *Phytophthora*-infected *Quercus lobata* seedings treated with hot water are shown in Figure 12. It took 5 to 8 min of continuous irrigation with 50-51 C water to get the soil temperature in the center of the containers above 47 C. We inadvertently exceeded the target temperature by more than intended on the first treated container (CAC3, blue line in figure 12). This container reached a maximum temperature of 51 C, the temperature of the added water. Soil in this container cooled very slowly in the 48 C water bath and was at 49 C when removed from the water bath. The two other containers were only slightly over the target temperature of 47 C. Total time at or above 47 C was 31 minutes for all three containers.

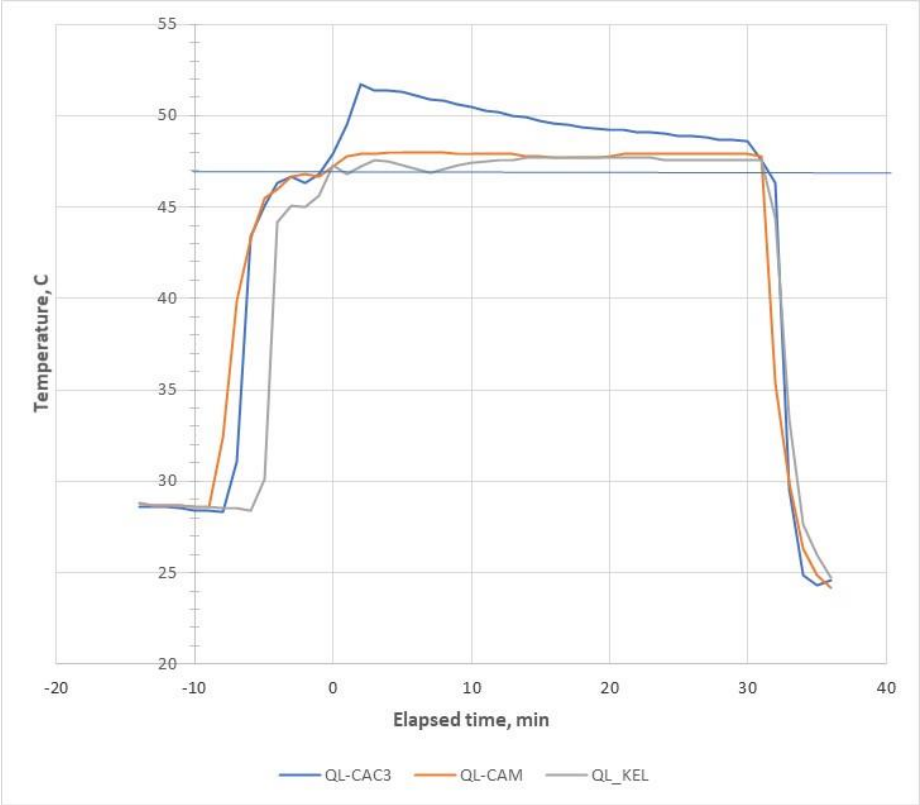

Figure 12. Test 10. Temperature traces for *Phytophthora* inoculated *Quercus lobata* in AB35 containers treated at target temperature 47 C for 30 min. Each plant was individually irrigated with hot (50-51 C) water until the temperature reached 48 C to compensate for heat loss in subsequent handling, but timing of the treatment started when the temperature reached 47 C. When the temperature reached 48 C, the containers was placed in a plastic bag and then into a 48 C water bath until the total elapsed time at 47 C or above was 30 minutes. Plants were removed from the water bath, unbagged, and irrigated with cool water until thermocouples measured 25 C.

No *Phytophthora* was detected by baiting leachate (12, 22, and 43 d after treatment) from the individual containers or in the final destructive baiting of the entire root/soil mass 93 days after treatment (Table 11). All heat-treated plants were live and did not show any obvious damage from the heat treatment (Figure 13). No new shoot growth was seen on either treated plants or noninoculated, nontreated controls over the evaluation period. No discoloration or evidence of *Phytophthora* infection was seen at the base of the stems when the outer bark was cut away (Figure 13). Varying amounts of healthy roots and new root growth were observed when the roots were examined (Figure 13). Few new (white) roots were observed on plants that had been inoculated with *P. cactorum* or *P. ×cambivora*, but more were observed on the plant inoculated with *P. kelmanii*. Most roots of both treated seedlings and noninoculated, nontreated control plants were mostly brown. Overall live root density in the control plant was greater overall than the treated plants, which may have lost roots due to *Phytophthora* prior to treatment. The heat treatment may also have delayed or reduced new root growth or caused some root death.

Table 11. Summary table showing detection of *Phytophthora* from inoculated *Quercus lobata* (QL) and *Q. agrifolia* (QA) by individual leachate baiting with green pears before and after heat treatment (tests 10 and 13). *Phytophthora* species used were two different isolates of *P. cactorum* (CAC3 and CAC4), *P. cambivora* (CAM), and *P. kelmanii* (KEL).

Host-pathogen	Before heat treatment: Days after inoculation: 4-5 d	9-10 d	15 d	Days from inoculation to treatment	Days from last leachate test to treatment	Days after heat treatment: 12 d	22 d (QL) 28 d (QA)	43 d	93 d (QL) 82 d (QA)
Test 10	***Phytophthora* detected (+) or not (–)**					***Phytophthora* detected (+) or not (–)**			
QL-CAC3	+	–	+	27	12	–	–	–	–
QL-KEL	+	+	+	18	3	–	–	–	–
QL-CAM	–	–	+	26	11	–	–	–	–
Test 13									
QA-CAC4	+	–	+	38	23	–	–	–	–
QA-KEL	+	+	+	29	14	–	–	–	–
QA-CAM	+	–	+	37	22	–	–	–	–

Figure 13. Test 10. Top-*Phytophthora* inoculated *Quercus lobata* in AB35 containers treated at target temperature 47 C for 30 min at 93 days after treatment (L to right: CAC3, CAM, KEL). Bottom left – bark and xylem tissues at the base of all seedlings was nondiscolored and healthy in appearance (QL-CAM shown). Bottom right – root ball of QL-KEL, showing apparently healthy roots.

Test 13 – *Phytophthora*-infected *Quercus agrifolia* seedlings treated at 47 C for 30 minutes

Test 13 was conducted in the same manner as test 10 except that the test plants were three inoculated *Quercus agrifolia* plants. During the pour-through of hot water, all plants exceeded the target temperature by about 1 C. One plant (QA-CAC4) cooled below the target by about 1 C during transfer to the water bath and remained slightly below 47 C for about 6 minutes (Figure 14). For this container total time treatment time was 31 minutes, including 6 minutes between 46.5 and 46.8 C. The other two pots stayed slightly above 47 C for the duration of the treatment (Figure 14). Total time at or above 47 C was 32 minutes for QA-CAM and 33 minutes for QA-KEL.

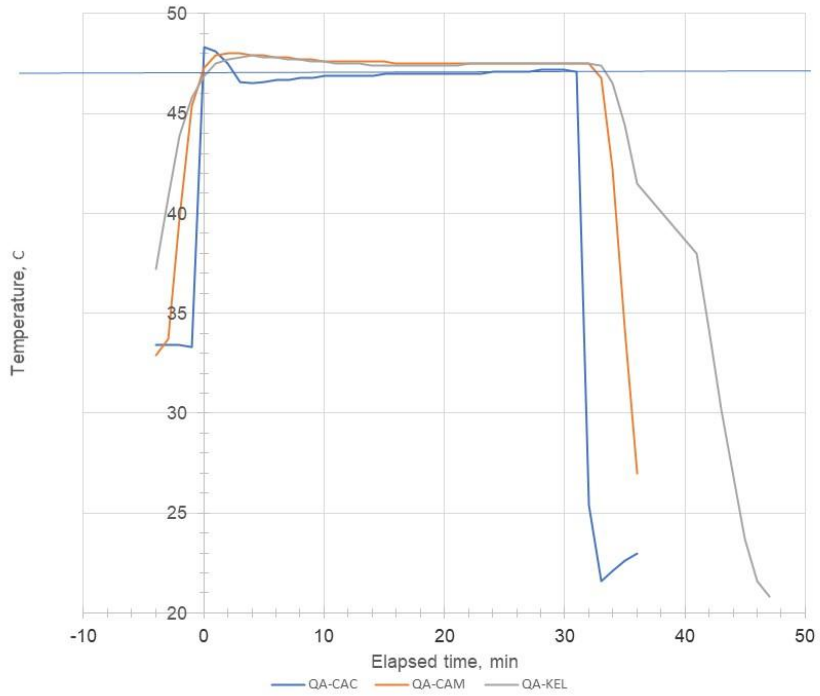

Figure 14. Test 13. Temperature traces for *Quercus agrifolia* plants in AB35 containers treated at target temperature 47 C for 30 min. Each plant was individually irrigated with hot (50-51 C) water until the temperature reached 48 C to compensate for heat loss in subsequent handling, but timing of the treatment started when the temperature reached 47 C. When the temperature reached 48 C, the container was placed in a plastic bag and then into a 48 C water bath until the total elapsed time at 47 C or above was 30 minutes. Plants were removed from the water bath, unbagged, and irrigated with cool water until thermocouples measured 25 C.

No *Phytophthora* was detected by baiting of leachate (12, 28, and 43 d after treatment) from the individual containers or in the final destructive baiting of the entire root /soil mass 82 days after treatment (Table 11). All heat-treated plants were live and did not show any obvious damage from the heat treatment (Figure 15). As seen in the *Q. lobata* seedlings, neither the treated or noninoculated, nontreated control *Q. agrifolia* seedlings showed any new shoot growth. No xylem discoloration or stem cankers were observed on any of the treated (Figure 15) or control plants. Root density was low overall in both heat-treated and control *Q. agrifolia* seedlings, with all plants having similar root appearance and few new roots (Figure 15).

Figure 15. Test 13. Top-*Phytophthora* inoculated *Quercus agrifolia* in AB35 containers treated at target temperature 47 C for 30 min at 93 days after treatment (L to right: CAC4, CAM, KEL). Bottom left – bark and xylem tissues at the base of all seedlings was nondiscolored and healthy in appearance (QA-CAM shown). Bottom right – root ball of QA-CAM, showing a few new white roots.

Discussion

This objective of this initial study was to determine whether thermotherapy could be developed into a feasible methodology for eliminating *Phytophthora* from container stock. To accomplish this, we needed to determine how to apply precise temperature treatments to container plants. Additionally, we needed to determine time × temperature exposures that could be tolerated by plants and yet kill *Phytophthora* species in their roots and container media.

Minimum temperature regimes needed to kill *Phytophthora*

Experiments with infected pear epidermis and rhododendron leaf disks and infected plants (tests 10 and 13) established that the three test *Phytophthora* species we used could be killed by exposure to 47 C for as little as 20 minutes. We did not anticipate this based on our review of the literature (Table 1), which showed that longer minimum treatment times were required at this and higher temperatures. However, one of the studies in Table 1 (Juarez-Palacios et al. 1991) showed that 45 C for 20 min rendered inoculum in infested soil and colonized walnut twigs nonviable after 20 min (*P. cinnamomi)* or 30 min (*P. cactorum*). Although the results of Juarez-Palacios et al. (1991) seem to be an outlier in Table 1, they are consistent with our results. Another recent article (Baggio et al. 2021) reported that a 4 h treatment of aerated steam at 44 C applied the day after inoculation with *P. cactorum* greatly reduced disease incidence of field-planted strawberry plants, suggesting that this was an effective time × temperature treatment.

The heat exposure times reported in Juarez-Palacios et al. (1991) were the time that the test materials were left in the 45 C water bath rather than actual temperatures measured in the bagged materials. Based on lag times that occurred in our tests when materials are placed in hot water (Figures 2-12), it is likely that the actual time that the treated materials were at 45 C would have been less than reported and it is possible that the target 45 C temperature was not attained throughout the treated material.

In multiple tests, we showed that the temperature of a water bath and the time that a sample was in it did not correspond well to the actual time × temperature regime experienced by the sample. As we illustrated here, it can take a considerable amount of time for the temperature in a container of material being treated to equilibrate with the temperature of a water bath. This applied to the soil and roots of container plants as well as disks of infected tissue in test tubes. Because water transfers heat much more efficiently than air, the lag before temperature equilibration occurs is likely to be even greater if the container with a plant or *Phytophthora* inoculum is placed in a heated chamber. Furthermore, we found that evaporative cooling from the surface of the treated material lowers the effective treatment temperature. We were able to compensate for this somewhat by maintaining the level of the water bath well above the surface of the material being treated (containers or water in test tubes). The processes of heat transfer and loss typically result in reduced temperatures and shorter exposure times than would be inferred from time spent in a water bath or temperature chamber.

When we rechecked the references we had reviewed for tables 1 and 2, we found that none of these reported temperature and exposure times for the target sample (plant or inoculum); only temperatures and exposure periods were reported. Hence, the actual temperatures and treatment durations that the samples attained in these studies are likely to be less than reported. Especially for thermotherapy, which requires precise control of temperature exposures, it is imperative that studies provide data on actual temperatures achieved in the target treated material, not just the temperature of the water bath or the temperature of the growth chamber. Based on the literature we have reviewed to date, this critical information is rarely reported, making it difficult to impossible to make valid comparisons between studies reporting temperature effects on *Phytophthora* survival. The net effect is that the literature tends to overestimate the minimum temperature × time required to kill *Phytophthora* inoculum. This is less of a problem for

phytosanitary purposes where the aim is to kill the pathogen because it provides some margin for error by overtreating the material. However, for thermotherapy, it is necessary to tread a fine line between what kills the pathogen and what kills the plant as well. If the temperature × time required to kill *Phytophthora* is overestimated, potentially effective treatment regimes will be overlooked.

Applying thermotherapy to container plants

In our experiments, we found that thermotherapy is difficult to accomplish with container plants due to both methodological and biological limitations. Because there is a small margin between heat treatments that may be therapeutic and those that are lethal to plants, treatments need to be fairly precise with respect to both the target temperature attained and the treatment duration. However, we found that it is difficult to apply precise heat-treatments to container plants due to the difficulty in raising the temperature of roots and soil quickly and uniformly throughout the container. The problem increases with the container volume.

The hot water pour-through method used in the tests 10 and 13 was the best method we devised to quickly raise the temperature of soil and roots in a container in a precise manner. By switching to cool water, this technique was also useful for quickly lowering soil and root temperature. However, this method is subject to variation based on the drainage rate of the containers. This fact required that each plant be treated and timed individually. If drainage is restricted by high root density or slow-draining media, this technique will not be satisfactory. It is also unlikely to provide uniform temperatures in much larger containers due to concurrent cooling processes, such as evaporative cooling of the soil surface.

Based on these considerations, we believe that the most precise way to apply thermotherapy treatments to container plants is to remove them from the containers, remove as much potting media as possible, and treat the exposed roots directly in a circulating water bath. The bare-root method could be scaled to larger plants, but treating large root systems would involve a number of other technical and logistical issues that could affect feasibility. Another alternative may be the use of used aerated steam, as used by Baggio et al (2021) to treat bare-root strawberry plants. However, the equipment for producing aerated steam is more expensive than that needed for hot water and it would be more difficult to heat-treat only the roots of plants when using aerated steam compared to water.

These initial experiments indicate that thermotherapy may have some very limited utility for treating *Phytophthora*-infected plants and is most likely to be successful for small plants and propagules. Working out the details for a viable thermotherapy treatment is somewhat time-consuming because both *Phytophthora* species and plants can vary in their thermotolerance, and the details of the heat treatment system may need to be adjusted based on plant size and other factors. Consequently, future work on thermotherapy should start with a particular host-pathogen disease situation for which thermotherapy provides the best or only viable option. For restoration nurseries, this might include situations where field-collected plants or propagules of rare species or genotypes need to be conserved but materials may be infected with *Phytophthora*.

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