Soil Treatments for the Potential Elimination of *Phytophthora ramorum* in Ornamental Nursery Beds

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ABSTRACT

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Ramorum leaf blight, caused by *Phytophthora ramorum*, has reemerged at several California nurseries after removal of infested material. In many cases, reemergence was not associated with reintroduction of the pathogen and may be attributed to inoculum surviving in soil beds because *P. ramorum* propagules can survive for over a year in soil. Using artificially infested soil in microcosms, fumigation and heat treatments were examined as potential eradicants of *P. ramorum* from soil. Treatments with chloropicrin, Vapam, and iodomethane were effective in reducing *P. ramorum* propagules below detection limits. Basamid was consistently effective only when fully incorporated into the soil. Application of Basamid (392 kg/ha) at infested ornamental nursery sites mirrored results from microcosm experiments, indicating that a tarp cover over treated soil is necessary for reliable efficacy. Dimethyldisulfide, 1,3-dichloropropene, and two formulations of hydrogen dioxide were less effective, resulting in only partial reduction of propagules. In heat treatments, *P. ramorum* in soil microcosms remained detectable 42 days after microcosms were incubated at 30 and 22°C but was not detectable in soil heated above 40°C for 3 days. Results from a solarized field plot indicate that prolonged sublethal temperatures, between 35 and 40°C for 42 days, can be effective in eliminating detectable propagules of *P. ramorum*.

Phytophthora ramorum is thought to be a pathogen introduced to the west coast of the United States and, prior to its discovery as the cause of tree mortality in coastal California woodlands, it had been reported as a foliar pathogen of ornamental *Rhododendron* and *Viburnum* spp. in Germany and the Netherlands (20,24). Awareness that these plants served as hosts in Europe led to examination of ornamental plants in areas of California infested with *P. ramorum.* It was determined that *Rhododendron* spp. and many other plants were serving as hosts to *P. ramorum* (2).

Growing awareness of the wide host range of *P. ramorum* and evidence of the pathogen in nurseries caused concerns about the possibility of spreading the pathogen with the movement of ornamentals. California is the leading producer of ornamental plants in the United States and ships plants throughout North America (4). In an attempt to limit the potential spread of *P. ramorum* to other regions, the United States Department of Food and Agriculture–Animal and Plant Health Inspection Service (APHIS) issued the "Order Restricting Movement of Nursery Stock from California Nurseries" in April 2004 (3). This was followed in October, 2004 by the APHIS "Official Regulatory Protocol for Nurseries Containing Plants Infected with *Phytophthora ramorum* (Sudden Oak Death)" (2). This "Confirmed Nursery Protocol" delineated monitoring and sanitation procedures for wholesale ornamental nurseries shipping plants outside of their county boundaries. The APHIS procedures call for inspections, *P. ramorum*-free certification, destruction of infested plants, and the elimination of *P. ramorum* from soil or gravel beds where container-grown plants might be placed.

P. ramorum has been shown to survive for extended periods in soil, indicating that effective remediation of nurseries harboring the pathogen will require thorough disinfestation of soil media and soil beds. In artificially infested potting media or sand, several studies indicate that propagules of *P. ramorum*, either fully exposed or imbedded in leaf tissue, can remain viable for a year or more (16,21,23). Baiting tests conducted in a California production nursery in 2005 following removal and destruction of an infected *Camellia* crop detected *P. ramorum* propagules in soil collected from the underlying nursery beds for more than 75 days after removal of plants and plant debris (26).

Agricultural systems have commonly used fumigation to manage soilborne pests (1,10,11,18,25). Hence, when the need arose to eliminate *P. ramorum* from infested nursery soils, APHIS specified several chemical treatment materials in the Confirmed Nursery Protocol. These included chloropicrin, dazomet, metam-sodium, and methyl bromide. Unfortunately, these treatments are problematic in many nursery production situations. Many nurseries are situated within urban centers and cannot accommodate the required buffer zones between fumigation sites and schools or homes, or they have small, inaccessible spaces which are not conducive to conventional soil fumigation equipment. Other problems involve township caps on fumigants that force nursery production to compete with other agricultural crops for use of the material, and the phase-out of methyl bromide. Additionally, container nurseries do not contain typical field soils that are compatible with conventional shank fumigation equipment. Nursery beds under potted plant stock commonly consist of packed gravel over soil, with gravel layers 15 cm or more in depth. Moreover, the efficacy of the APHIS-recommended soil treatments has not been determined for *P. ramorum* in the nursery environment. For example, monitoring a nursery treated with Basamid (392 kg/ha) and the label-recommended "water barrier" over the soil surface following application showed that the label treatment did not eradicate *P. ramorum* (26). Finally, there are additional fumigants available or under development such as dimethyldisulfide and iodomethane that might be suitable for nurseries but were not included as options in the APHIS listing of approved materials.

The Confirmed Nursery Protocol does indicate that heat can be used as an alternative to soil fumigation. Soil solarization and steam treatments have been successful in controlling other plant pests in other production systems but little information is available to guide the use of these technologies in container nursery beds (5–8,17,19). The main limiting factor in using heat for soil bed sanitation is the ability to deliver heat through the soil profile. Dart et al. (9) were able to isolate *P. ramorum* down to 10 cm in several commercial nurseries in Washington. In California, *P. ramorum* could be detected at depths down to the hard pan ranging from 15 to 45 cm (26). The temperatures attained at different soil depths via steam and solar treatments are highly influenced by solar radiation intensity, soil moisture, and soil texture and density (15).

This study was initiated to examine options for elimination of *P. ramorum* from nursery beds. Chemical and nonchemical

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approaches were included to provide more treatment options for affected nurseries. It is hoped that this work will give growers greater flexibility and reliability as they attempt to treat nursery areas infested with *P. ramorum*.

MATERIALS AND METHODS

Microcosms. Quarantine restrictions precluded experiments with *P. ramorum* at commercial nurseries or experimental lands. Therefore, microcosms were employed in a controlled laboratory setting to evaluate experimental treatments for control of *P. ramorum*. Most fumigation experiments were conducted in glass Mason jars (Ball, Muncie, IN) of approximately 950-ml capacity whereas heat treatments were tested in smaller glass Mason jars of approximately 118 ml to maximize the rate of heat transfer to the contained soil. Chemicals added as soil drenches were tested in open-ended cone-tainers (3.8 cm in diameter by 21 cm long) (Ray Leach Cone-tainer Nursery, Canby, OR) or in glass cylinders (11.5 cm in diameter by 45 cm long) to allow for bottom drainage. All microcosms were incubated for various times at approximately 22°C in a fume hood.

Inoculum and soil. Twelve California isolates of *P. ramorum*, A2 mating type, were used to infest the Yolo fine sandy loam used in these experiments. The soil had a mean particle size distribution of 33% sand, 47% silt, and 20% clay. The pH was 7.0 and the organic C content was 0.99%. Eleven isolates were obtained from the *P. ramorum* culture collection of D. M. Rizzo, University of California, Davis. They were isolated originally from foliar, stem, or root lesions found on *Rhododendron* spp., California bay laurel (*Umbellularia californica*), tanoak (*Lithocarpus densiflorus*), or *Camellia* spp. found in ornamental nurseries or forests. An additional isolate was from soil under infested *Camellia* stock in a commercial nursery.

To infest soil, two plates of each isolate were grown for 2 weeks at 22°C on V8 agar medium (14). Cultures were microscopically examined to ensure the presence of chlamydospores. Sporangia were also present for some isolates. After 2 weeks, the uncolonized agar was removed and disposed of. The remaining colonized agar was homogenized in a blender for 30 s with 500 ml of sterile, distilled water. The resulting slurry was incorporated into 70 kg of the aforementioned soil that had been autoclaved three times for 60 min over a 72-h period. One to two infestations occurred until a population level of approximately 120 CFU of *P. ramorum* per gram of dry soil was achieved. Concurrently with infestation, sterile distilled water was added to the soil to achieve a final desired soil moisture content of 20% (vol/vol). Soil moisture content was assessed by drying an aliquot of soil in a 121°C oven for 48 h. The moistened, infested soil was thoroughly mixed by hand and allowed to incubate for at least 48 h before being packed into microcosms. Soil was packed into the microcosms at a bulk density of approximately 1 g/ml and a soil column height of approximately 7.5 cm. Infestation levels were verified by dilution plating on CMA-PARP medium as described below.

Liquid fumigants. Individually and in combination, chloropicrin, 1.3-dichloropropene (1,3-D), iodomethane, dimethyldisulfide (DMDS), and metam sodium (Vapam) were first tested at recommended rates using three replications in each of two separate trials. Depending on the ability of the fumigant to eliminate *P. ramorum* propagules, half rates were then tested in triplicate in two separate trials. Fumigant rates were as follows: 1,3-D (112 kg/ha), chloropicrin (56 and 112 kg/ha), DMDS (224 kg/ha), chloropicrin:DMDS (112:224 kg/ha), iodomethane (56 and 112 kg/ha), iodomethane:chloropicrin (56:112 and 112:224 kg/ha), Telone C35 (Dow AgroSciences, Indianapolis, IN), a formulation of 1,3-D and chloropicrin, (370 and 739 liters/ha), and Vapam (a formulation of metam sodium; Amvac, Los Angeles) (350 and 700 liters/ha).

Fumigants were added by injection using a gas-tight, glass syringe (Hamilton Co., Reno, NV) at the surface of the soil column. Injection always occurred at the center of the soil column diameter. Negative controls consisted of infested soil left untreated. The open mouths of the microcosms were covered with 1-mil polyethylene plastic that was secured by rubber bands. The plastic was removed to vent the fumigant 14 days after fumigation or at the minimum restricted entry interval recommended (REI) by the label, which is 5 days after fumigation with chloropicrin, iodomethane, and Telone C35 and 2 days after fumigation with Vapam. All microcosms were allowed to vent for 7 days before sampling. Microcosms were incubated in a completely randomized design in a fume hood at room temperature (22°C).

Basamid. The efficacy of Basamid (Certis, Columbia, MD), a granular formulation of dazomet, was tested by three methods. In the first method, Basamid granules (112 and 224 kg/ha) were mixed uniformly into the infested soil before being packed into the 950-ml microcosms. Sterile, distilled water (10 ml) was added to the soil column before the microcosms were covered with 1-mil polyethylene. Negative controls consisted of infested soil treated only with 10 ml of sterile, distilled water and covered with polyethylene. The polyethylene plastic was removed to vent the fumigant after 14 days or 2 days, the REI recommended.

In the second method, Basamid granules (224 and 448 kg/ha) were sprinkled onto the surface of the soil column in a 950-ml microcosm. Sterile, distilled water (80 ml) was added to the surface to irrigate the soil and potentially spread the fumigant. Negative controls consisted of infested soil treated only with 80 ml of sterile, distilled water. The microcosms were covered with polyethylene tarp for 2 days.

In the third method, an open-ended glass cylinder was packed with a 3-cm gravel layer at the bottom of the cylinder followed by infested soil to create a soil column of 30 cm topped with an 8-cm layer of gravel. Basamid granules (224 and 448 kg/ha) were placed on the gravel surface and irrigated with 500 ml of sterile, distilled water. Negative controls consisted of soil columns constructed as described and only irrigated with 500 ml of sterile, distilled water. The soil columns were covered with polyethylene plastic. The polyethylene plastic was removed 2 days after fumigation.

All Basamid treatments were replicated three times in each of two trials. Microcosms were incubated in a completely randomized design in a fume hood at room temperature (22°C) and vented 7 days before sampling.

Soil drenches. The efficacy of labeled rates of Terraclean (1:1000 dilution; BioSafe Systems, East Hartford, CT) and Zerotol (1:50 dilution; BioSafe Systems), formulations of hydrogen dioxide, were tested by adding infested soil to 164-ml cone-tainers. The soil was packed to a bulk density of 1 g/ml. Terraclean or Zerotol (50 ml each) was added to the cone-tainers, a volume that just yielded drainage from the container bottom. The drenched tubes were incubated for 5 days in a fume hood. Negative controls were cone-tainers filled with infested soil and irrigated with 50 ml of sterilized, distilled water. Two trials of four replications were conducted for each treatment.

Heat treatments. To determine critical exposure temperatures and times for heat treatments of soil to eliminate *P. ramorum*, infested soil was packed into 118-ml jars to a density of 1 g/ml. The microcosms were sealed with the original Mason jar lids. In two replicates of each treatment, temperature probes (Onset, Sunnyvale, CA) were inserted through the lids to monitor soil temperatures. Microcosms were incubated in dark containers in incubators to achieve 30, 40, and 60°C or in a water bath to achieve 80°C. Untreated controls were incubated on the bench top at 22°C. Exposure times were 3, 7, 14, 28, 42, or 56 days, except at 80°C, where exposure times were 15, 45, and 75 min. Two trials of four replications each were conducted for all treatments.

Soil assays to detect survival. To test for survival of *P. ramorum* following the aforementioned chemical and heat treatments, aliquots of soil were suspended in water and plated onto CMA-PARP (14).

All soil was removed from each microcosm and thoroughly mixed in a plastic bag to homogenize the soil. Sterile, distilled water (100 ml) was added to 100 g wet weight of soil and vortexed to suspend the soil particles. Then, 1 ml of each dilution was spread over the surface of three pseudoreplicate plates. The solutions spread on the plates ranged from full-strength to 1:1000 dilutions. Plates were incubated at approximately 22°C in dark crispers for 3 weeks before the soil was washed off of the surface of the plates with water to count colonies. If no colonies were present, plates were incubated for an additional 2 weeks to check for delayed colony development.

Statistical analysis. Efficacies of treatments are reported as the percent reduction of *P. ramorum* propagules due to treatment compared with the appropriate untreated control. A one-way nonparametric analysis of percent reduction was required because the data did not meet the assumptions for parametric analysis. This analysis was conducted by first ranking the data using PROC RANK in SAS (version 9.2; SAS Institute, Inc., Cary, NC) and then subjecting the ranked data to an analysis of variance (ANOVA) with the PROC GLM procedure in SAS. Treatment means were separated using Tukey's w procedure ($P \leq 0.05$).

RESULTS

In microcosm experiments, chloropicrin and metam sodium (Vapam), materials recommended in the APHIS "Official Regulatory Protocol", in addition to iodomethane eliminated detectable inoculum of *P. ramorum* (Table 1). No colonies of *P. ramorum* were detected by culture plating after treatment with label-recommended rates or half rates of chloropicrin, Vapam, Telone C35, or iodomethane. There was no difference in efficacy whether plastic mulch was kept in place for 14 days or for the minimum reentry interval.

1,3-D and DMDS were only partially effective. At label rates, 1,3-D and DMDS reduced populations by 37 and 33%, respectively, compared with the controls. When 1,3-D and DMDS were combined with chloropicrin, *P. ramorum* was reduced below detection limits. Because 1,3-D and DMDS did not eliminate all inoculum, further experimentation with lower rates and fumigation times was not conducted with these materials.

No viable propagules were detected by culture plating after dazomet (Basamid) was mixed throughout the soil profile and tarped for 14 days or for the minimum reentry interval (Table 1). Additionally, no viable propagules were detected after Basamid was irrigated in from the soil surface of a 950-ml microcosm and tarped for 14 days or for the minimum reentry interval. However, when Basamid was irrigated in using the larger volume of soil in an open-cylinder microcosm, the treatment was statistically different from untreated controls ($P \leq 0.05$) but only reduced *P. ramorum* by 32% (Table 1).

Soil drenches with labeled rates of hydrogen dioxide (Terraclean and Zerotol) only slightly reduced populations of *P. ramorum* in soil (Table 1). When applied according to label directions, Terraclean and Zerotol reduced detectable *P. ramorum* populations by 15 and 21%, respectively. Because these chemicals showed little efficacy, further experimentation was not conducted.

CFU of *P. ramorum* were eliminated at 80, 60, and 40 but not at 30 and 22°C (Table 2). At 40 and 60°C, the pathogen was not detected in soil that had been incubated at these temperatures for 3 days or more. At 80°C, propagules were not detected after 15 min, the minimum exposure time tested. Viable propagules of *P. ramorum* in soil incubated at 30°C were significantly different from untreated controls, declining slightly by 33 over the 7-day incubation period.

Table 1. Reduction of propagules of *Phytophthora ramorum* due to treatments with fumigants and chemical drenches[w]

Chemical	Rate	Fumigant placement	Tarped period (days)	Reduction (%)[x]
1,3-Dichloropropene	112 kg/ha	Top	14	37 b
	224 kg/ha	Mixed throughout	14	100 a
	112 kg/ha	Mixed throughout	1	100 a
	224 kg/ha	Mixed throughout	1	100 a
Basamid	224 kg/ha	Top + 80 ml of water	2	100 a
	448 kg/ha	Top + 80 ml of water	2	100 a
	224 kg/ha	Top + 500 ml of water[y]	2	37 b
	448 kg/ha	Top + 500 ml of water[y]	2	49 b
Chloropicrin	112 kg/ha	Top	14	100 a
	112 kg/ha	Top	5	100 a
	56 kg/ha	Top	5	100 a
Dimethyldisulfide	224 kg/ha	Top	14	33 b
Chloropicrin:dimethyldisulfide	112:224 kg/ha	Top	14	100 a
Iodomethane	112 kg/ha	Top	14	100 a
	112 kg/ha	Top	5	100 a
	56 kg/ha	Top	5	100 a
Iodomethane:chloropicrin	112:224 kg/ha	Top	14	100 a
	112:224 kg/ha	Top	5	100 a
	56:112 kg/ha	Top	5	100 a
Telone C35	739 liters/ha	Top	14	100 a
	739 liters/ha	Top	5	100 a
	370 liters/ha	Top	5	100 a
Vapam	700 liters/ha	Top	14	100 a
	700 liters/ha	Top	2	100 a
	350 liters/ha	Top	2	100 a
Terraclean	1:1000 dilution	Drench[z]	…	15 b
Zerotol	1:50 dilution	Drench[z]	…	21 b

[w] Treatments were conducted in 950-ml microcosms unless otherwise specified. If applicable, 1-mil polyethylene plastic was secured to the mouth of the microcosms to simulate a tarp.

[x] Values indicate the percent reduction of propagules in relation to the appropriate negative control. Statistically significant differences as determined by Tukey's w procedure are indicated by different letters ($P \leq 0.05$).

[y] Treatments were conducted using an open-ended cylinder which contained a soil profile of 30 cm, which was 22.5 cm longer than that contained in the 950-ml microcosms.

[z] Treatments were conducted in open-ended cone-tainers. The drench was added until drainage occurred from the bottom of the cone-tainer (50 ml).

Table 2. Reduction of propagules of *Phytophthora ramorum* in soil due to temperature and temperature exposure time[z]

Temperature (°C), time	Reduction (%)
30	
7 days	35 ab
14 days	22 ab
28 days	27 ab
42 days	37 b
56 days	22 a
40	
3 days	100 c
7 days	100 c
14 days	100 c
28 days	100 c
42 days	100 c
56 days	100 c
60	
3 days	100 c
7 days	100 c
14 days	100 c
28 days	100 c
42 days	100 c
56 days	100 c
80	
15 min	100 c
45 min	100 c
75 min	100 c

[z] Treatments were conducted in 180-ml microcosms. Values indicate the percent reduction of propagules in relation to the negative controls which were incubated at 22°C. Statistically significant differences as determined by Tukey's w procedure are indicated by different letters ($P \leq 0.05$).

DISCUSSION

In these experiments, applications of the soil fumigant materials recommended in the Confirmed Nursery Protocol—chloropicrin, dazomet, and metam sodium—applied at recommended rates and used in combination with tarping effectively eliminated detection of *P. ramorum* propagules in soil. Even half label rates combined with a tarping period equal to labeled minimum reentry intervals were effective. These data suggest that nurseries that can accommodate conventional fumigation methods and regulations can expect to eliminate *P. ramorum* in their nursery beds.

Serving as examples, three monitored California nurseries successfully eliminated detectable *P. ramorum* from their infested beds. In all cases, Basamid (392 kg/ha) was tilled into the infested soil and the treated areas were then tarped for 2 weeks. After a week of venting, *P. ramorum* was not detected by baiting tests in soil from the treated areas (26). Follow-up samples were taken the following year, and *P. ramorum* still could not be detected in any of the treated areas.

Although Basamid was effective when tilled into the soil and tarped according to label directions, many nurseries cannot utilize this method because their nursery beds contain large quantities of gravel. The thick layer of coarse gravel precludes tilling and forces reliance upon the application of Basamid through water infiltration into the soil profile. According to our laboratory experiments, however, *P. ramorum* can be eliminated using this water infiltration method only in small volumes of soil. Although propagules of *P. ramorum* were not detectable in the 7.5-cm (950-ml) microcosm, propagules were detectable in the 30-cm open-ended microcosm. One California commercial nursery was successful in using the water infiltration application of Basamid. However, although the beds were covered with 10 cm of course gravel, the underlying soil was very shallow (2 to 3 cm) and underlain by a severely compacted mineral layer. Therefore, the results at this nursery site may not be representative (L. E. Yakabe, *unpublished*).

1,3-D, DMDS, and iodomethane were also tested in these experiments, even though the materials were not included in the Confirmed Nursery Protocol recommendations. Iodomethane, a component of Midas (Arysta LifeScience North American Corporation. Cary, NC), is currently undergoing registration in California. Iodomethane effectively eliminated detection of *P. ramorum*. In contrast, both 1,3-D and DMDS failed to eliminate *P. ramorum* from the microcosms. 1,3-D, a fumigant mainly used for its nematicidal qualities, is effective in combination with chloropicrin. However, as in other studies, 1,3-D has decreased efficacy against fungal and oomycete organisms when used alone (12). DMDS is a fumigant currently in development and is still unregistered.

Hydrogen dioxide in the formulations of Zerotol and Terraclean was ineffective in these trials. Their chemical characteristics make them unlikely to be an effective eradicant in the soil environment even though they are labeled for soil drenches. Peroxides kill through their oxidizing properties. However, because there are many nontarget substances that can be oxidized in soil, the oxidizing potential is quickly consumed by nontarget substrates. In addition, soil drenches work by contact rather than volatilization and, hence, are inherently less well distributed through the soil profile.

Nurseries unable to use fumigation can consider heat treatments as an alternative for nursery bed remediation. Swain et al. (22) established that a minimum of 40°C for 24 h is lethal to cultures of *P. ramorum*. Transferring these results into soil, these experiments indicate that *P. ramorum* was not detectable in soil treated with temperatures greater or equal to 40°C for 3 days. A maximum exposure time of 15 min is required for elimination of detectable *P. ramorum* if soil temperatures of 80°C can be achieved. These results are consistent with research conducted in sand and potting media. Tooley and Browning (23) reported that chlamydospores were inactivated in sand when incubated at 40°C for as little as 24 h. Chlamydospore viability steadily declined when incubated at 35°C and eventually were completely inactivated after 7 days. If embedded in plant tissue, chlamydospores required a 2-day exposure at 40°C or a 4-day exposure at 35°C for inactivation. Using aerated steam in potting media, Linderman and Davis (16) were able to eliminate *P. ramorum* propagules at temperatures greater than 50°C when maintained for 30 min. Harnik et al. (13) reported that intact bay leaves infested with *P. ramorum* required 2 weeks of 55°C to eliminate *P. ramorum* detection. For these reasons, efforts to remove large plant debris should be taken before heat treatment.

A single, small solarization trial was conducted at a field site used by a University of California Cooperative Extension Advisor in Felton, CA. Plant debris was removed and the infested ground was divided into four treated and four untreated plots. The treated plots were solarized using a 1-mil clear tarp for 6 weeks. Temperature probes were inserted into the soil at depths of 5, 15, and 30 cm. Maximum temperatures recorded at these depths were 40, 31, and 26°C, respectively. Before solarization, *P. ramorum* was detected by soil baiting with *Rhododendron* leaves throughout the soil profile down to 30 cm. *P. ramorum* was not detectable by soil baiting at the end of the trial in the solarized plots but was detectable in the untreated plots. These results suggest that prolonged exposure to sublethal temperatures of below 40°C may eventually eliminate propagules of *P. ramorum*.

Unevaluated soil treatments recommended in the Confirmed Nursery Protocol have made remediation of *P. ramorum*-infested nurseries difficult and unreliable. Although the quarantine status of *P. ramorum* has made controlled field trials infeasible, in vitro evaluations provide evidence that the Confirmed Nursery Protocol recommendations can be effective. Iodomethane, a fumigant not included in the recommendations, is also effective. Basamid efficacy is dependent on proper application. For reliable efficacy, Basamid should be fully incorporated into the soil and then tarped. Nurseries unable to fumigate must rely on heating technologies. Steam sterilization and solarization can be effective if adequate heat can be delivered to *P. ramorum* propagules. In conjunction with data from other studies, experiments indicate that soil temperatures of at least 40°C should be sustained for several days. If higher temperatures are achieved, reliability will be increased and less treatment time will be required. The effects of common nursery substrates such as gravel layers and compacted soil remain uncertain in both fumigation and heat treatments. Further experimentation should be conducted under field conditions to adequately explore remediation treatments.

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