Effects of Soil Solarization and *Trichoderma asperellum* on Soilborne Inoculum of *Phytophthora ramorum* and *Phytophthora pini* in Container Nurseries

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Abstract

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Infested container nursery beds are an important source of soilborne *Phytophthora* spp. for initiating disease through movement with surface water or splashing onto foliage. We investigated the effects of soil solarization, alone or with subsequent amendment with a *Trichoderma asperellum* biocontrol agent, on the survival of *Phytophthora* spp. inoculum. In field trials conducted with *Phytophthora ramorum* in San Rafael, CA and with *P. pini* in Corvallis, OR, infested rhododendron leaf inoculum was buried at 5, 15, and 30 cm below the soil surface. Solarization for 2 or 4 weeks during summer 2012 eliminated recovery of *Phytophthora* spp. buried at all depths in California trial 1, at 5 and 15 cm in California trial 2, but only at 5 cm in Oregon. There was no significant reduction of *Phytophthora* spp. recovery after *T. asperellum* application. Although the population densities of the introduced *T. asperellum* at the 5-cm depth were often two- to fourfold higher in solarized compared with nonsolarized plots, they were not significantly different ($P = 0.052$). Soil solarization appears to be a promising technique for disinfesting the upper layer of soil in container nurseries under certain conditions.

The quarantine pathogen *Phytophthora ramorum* Werres, De Cock & Man in't Veld, causal agent of sudden oak death (SOD), has killed over 1 million trees in coastal California and southwest Oregon in the United States. The pathogen is spread by movement of nursery plants (Goss et al. 2009), and there were a total 561 nursery detections in the United States between 2001 and 2014 (United States Department of Agriculture–Animal and Plant Health Inspection Service [USDA-APHIS]). Once infested by the pathogen, nursery beds are difficult to disinfest and many individual nurseries are recurrently positive.

P. ramorum can survive for extended periods in soil (Linderman and Davis 2008; Shishkoff 2007; Tooley et al. 2008; Vercauteren et al. 2012). Vercauteren et al. (2012) showed that *P. ramorum* could be recovered from infested soil for at least 33 months under field conditions. Therefore, effective remediation of nurseries infested by the pathogen will require thorough disinfestation of potting media and soil. Historically, soil fumigants have been applied to disinfest nursery beds; however, recent restrictions on the use of soil fumigants require the development of alternative methods.

Soil solarization has been used for managing many soilborne plant pathogens (Gamliel and Katan 2012). Solarization employs solar radiation to heat the soil under a transparent plastic film to achieve temperatures that are detrimental to soilborne pathogens. Previous studies have demonstrated that soil solarization is effective against some *Phytophthora* spp. (Juarez-Palacios et al. 1991; Porras et al. 2007), and a preliminary study investigated the effects of solarization on *P. ramorum* (Yakabe and MacDonald 2010). Although *P. ramorum* chlamydospores were killed by exposure to 30 min at 50°C (Linderman and Davis 2008), 2 days at 40°C, and 4 days at 35°C (Tooley et al. 2008), it was not known whether solarization could achieve temperatures high enough to kill *P. ramorum*.

Most solarization studies have been conducted in warmer climate areas, such as California and Israel. The Pacific Northwest has a cooler climate but there are some reports of successful solarization of *Phytophthora* spp. in field soil (Pinkerton et al. 2000, 2002, 2009). Temperatures attained with solarization at different soil depths are highly influenced by soil physical properties such as soil moisture, soil texture, and bulk density (Gamliel and Katan 2012). Little information is available to guide the use of soil solarization in container nursery beds, where soil is usually very compacted and gravelly. Moreover, advances in the development of horticultural plastic films with anticondensation coatings may increase the potential for solar heating; solarization effectiveness with these newer films has not been reported.

Trichoderma spp. have been shown to be effective biocontrol agents of soilborne fungi (Benítez et al. 2004; Harman 2006) and plant-parasitic nematodes (Affokpon et al. 2011). They also have been shown to inhibit the growth of *Phytophthora* spp. and reduce the diseases caused by them (Harman 2000; Kelley and Rodriguez-Kabana 1976; Porras et al. 2007; Smith et al. 1990; Washington et al. 1999). Porras et al. (2007) reported that the combination of solarization and *Trichoderma* spp. was effective in reducing *P. cactorum* (Lebert & Cohn) J. Schröt soil populations. *Trichoderma asperellum* isolate 04-22 was reported to be an effective biocontrol agent against soilborne *P. ramorum* chlamydospores (Widmer 2014). Because solarization has previously been shown to improve the establishment of microorganisms introduced subsequently (Jayaraj and Radhakrishnan 2008), we sought to determine whether solarization could improve the establishment of *T. asperellum* and provide biocontrol of *P. ramorum.*

The objectives of this research were to evaluate the potential use of soil solarization for eradicating *P. ramorum* in the surface soil of nursery beds in California and Oregon, and to evaluate its effect on establishment of the *T. asperellum* biocontrol agent. Because *P. ramorum* is a quarantine pathogen, field trials with *P. ramorum* were allowed only in the National Ornamentals Research Site at Dominican University of California (NORS-DUC) facility. In the Oregon field trial, we substituted *P. pini* Leonian as a surrogate for *P. ramorum*. *P. pini* (previously *P. citricola* Sawada) is also a destructive plant pathogen in container nurseries but has been reported to tolerate higher temperatures than does *P. ramorum* (Jung and Burgess 2009; Werres et al. 2001).

Materials and Methods

Field experiments. Three field trials with a similar experimental design were conducted in two sites: at NORS-DUC, San Rafael,

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CA, and the Oregon State University Botany Farm and Field Lab, Corvallis. Two trials were conducted in California with *P. ramorum*: trial 1 began 22 July 2012 and trial 2 began 17 August 2012. One trial was carried out with *P. pini* in Oregon, beginning 24 August 2012.

The soil at the California site was a sandy "fill" (sand 55.2%, silt 25.2%, clay 19.6%; gravel fraction 27.0%, bulk density 1.85 g/cm^3, pH 6.4, 4.7% organic matter). Soil in Oregon was a Camas gravelly sandy loam (sand 57.4%, silt 27.1%, clay 15.5%; gravel fraction; 12.2%, bulk density 1.93 g/cm^3, pH 6.5, 2.6% organic matter).

The experimental design for each trial was a split plot, with solarization as the main plot and biocontrol amendment as the subplot. Each trial consisted of 12 main plots, each 2.5 by 2.5 m, and treatments were arranged in a randomized complete block design with six replications per solarization treatment. Plots in the solarized treatment were each covered with a transparent plastic sheet and plots in the nonsolarized treatment were left uncovered. Subplots for subsequent amendment with a *Trichoderma* biocontrol treatment were established by dividing each main plot into two subplots. After the initial solarization treatment period, a *T. asperellum* biocontrol agent was applied to one of the subplots in each main plot (TA) and the other subplot was not amended (non-TA).

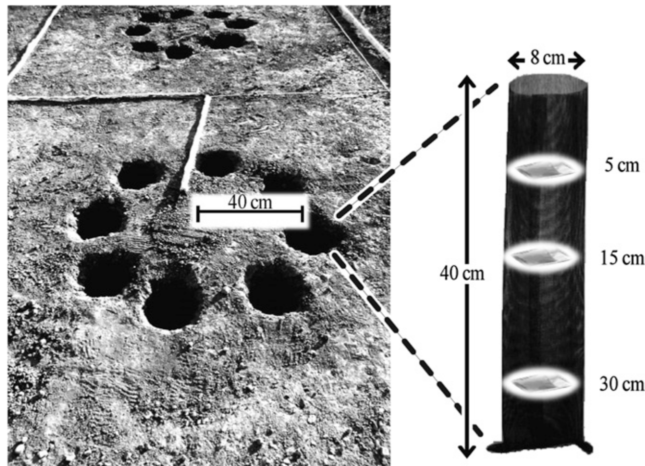

Fig. 1. Arrangement of eight soil-filled columns in each plot. Mesh bags containing leaf disk inoculum were placed in the columns at depths corresponding to 5, 15, and 30 cm below the soil surface. Soil water content reflectometers (not shown) were placed at the center of each plot at depths of 5, 15, and 30 cm for recording soil temperature, volumetric water content, and bulk electrical conductivity.

***Phytophthora* spp. inoculum.** *P. ramorum* isolate Pr-1418886 was used in California and *P. pini* isolate Pc98-517 was used in Oregon to produce infested leaf disk inoculum. *P. ramorum* zoospores were produced according to established methods (Parke and Lewis 2007) but with dilute (one-third strength) V8 broth agar substituted for V8-CMA. *P. pini* zoospores were produced according to Ochiai et al. (2011). Rhododendron 'Catawbiense Boursault' leaves were collected from plants maintained in a greenhouse at Oregon State University. Leaves were dipped into a zoospore suspension (5 × 10^4 zoospores/ml) and incubated in a moist chamber at 20°C (*P. ramorum*) or 24°C (*P. pini*). After 2 weeks, 6-mm-diameter disks were removed from leaf lesions using a hole punch. The presence of chlamydospores (*P. ramorum*) or oospores (*P. pini*) within leaf tissue was confirmed by microscopic observation of cleared leaves prior to use as inoculum. Mesh bags (4 by 4 cm) were constructed from nylon phytoplankton netting (100-µm opening; Aquatic Ecosystems, Apopka, FL) and filled with sieved (<2 mm) soil from each site and 10 infested leaf disks. Eight columns (8 cm in diameter by 40 cm in depth) were prepared for each main plot (Fig. 1). Columns were made from plastic window screen material to allow for drainage and aeration. The columns were filled with field soil, and mesh bags with leaf disk inoculum and sieved soil were inserted at depths corresponding to 5, 15, and 30 cm below the soil surface. Columns were placed in cylindrical holes (12 cm in diameter by 40 cm deep) arranged in a circular pattern 40 cm from the center of each main plot. Soil water content reflectometers (CS655; Campbell Scientific. Logan, UT) were placed at depths of 5, 15, and 30 cm in the center of three main plots in each solarization treatment. Soil temperature, volumetric water content, and bulk electrical conductivity data were recorded every 30 min with a CR1000 datalogger (Campbell Scientific).

Field sites were irrigated to saturation and allowed to drain overnight, and initial samples were collected the next day. Plots in the solarization treatment were each covered by a 6-mil (0.15-mm-thick) anticondensation polyethylene sheet (Thermax; AT Films, Edmonton, Alberta, Canada). Edges of the plastic sheets were "sealed" and held in place by a 15-cm-wide layer of gravel along the margin. The nonsolarized plots were left uncovered. Solarization was conducted for 6 weeks in the California trials and 4 weeks in the Oregon

Table 1. Soil temperature summary during the first 4 weeks in solarized and nonsolarized plots in trial 1 (CA 1; 22 July to 19 August 2012) and trial 2 (CA 2; 17 August to 14 September 2012) in San Rafael, CA, and in Corvallis, OR (OR; 24 August to 21 September 2012)

			Average daily soil temperature (°C)[a]					Cumulative hours			
Trial	Soil depth	Treatments	Mean	(s.e.)	Max	Min	Amp	>30°C	>35°C	>40°C	>50°C
CA 1	5 cm	Solarized	37.0	(0.2)	51.5	27.4	24.1	440	302	215	76
		Nonsolarized	27.6	(0.3)	39.7	19.2	20.5	224	138	48	0
	15 cm	Solarized	35.2	(0.3)	40.9	30.8	10.1	570	307	97	0
		Nonsolarized	27.2	(0.2)	31.9	23.4	8.5	145	0	0	0
	30 cm	Solarized	32.9	(0.1)	34.8	31.5	3.3	553	130	0	0
		Nonsolarized	26.8	(0.2)	28.3	25.7	2.6	0	0	0	0
CA 2	5 cm	Solarized	34.4	(0.2)	47.8	25.1	22.7	464	312	210	7
		Nonsolarized	25.3	(0.2)	36.1	17.4	18.7	217	87	1	0
	15 cm	Solarized	33.0	(0.2)	38.4	28.3	10.1	591	232	8	0
		Nonsolarized	25.2	(0.2)	28.9	21.7	7.2	15	0	0	0
	30 cm	Solarized	31.0	(0.1)	32.3	29.5	2.8	652	0	0	0
		Nonsolarized	25.1	(0.2)	26.1	24.0	2.1	0	0	0	0
OR	5 cm	Solarized	30.2	(0.1)	40.8	22.3	18.5	340	198	78	0
		Nonsolarized	22.1	(0.1)	29.0	16.5	12.5	42	0	0	0
	15 cm	Solarized	29.1	(0.2)	32.8	25.7	7.1	277	17	0	0
		Nonsolarized	22.0	(0.0)	24.4	19.7	4.7	0	0	0	0
	30 cm	Solarized	27.8	(0.1)	29.0	26.8	2.2	57	0	0	0
		Nonsolarized	21.8	(0.0)	22.5	21.1	1.4	0	0	0	0

[a] Mean = mean daily soil temperatures, which were significantly different between solarized and nonsolarized treatments for each soil depth in each of the three trials, by Mann-Whitney U test ($P = 0.05$, $n = 3$); s.e. = standard error of the mean. Average maximum (Max) and minimum (Min) daily soil temperature and daily amplitude (Amp = Max − Min) for the first 4 weeks during solarization treatment.

trial. The mesh bags from the three depths in each main plot were retrieved after 4 and 6 weeks in California trial 1; after 2, 4, and 6 weeks in California trial 2; and after 2 and 4 weeks in Oregon. During the solarization period, samples from the solarized treatment were collected by making small cuts on the plastic sheets, retrieving samples, and sealing the cuts with clear tape. There was a minimal effect on soil temperature and moisture. After solarization treatments were concluded, the plastic sheets were removed, and subplots receiving the *Trichoderma* biocontrol agent were amended with *T. asperellum* isolate 04-22 inoculum at 84 g/m^2 (1.72×10^9 CFU/g) provided by Dr. Timothy Widmer, United States Department of Agriculture–Agricultural Research Service (USDA-ARS, Ft. Detrick, MD). Inoculum consisted of a dried conidial powder produced by a proprietary process that was added to the plots to achieve a final concentration of 1×10^7 CFU/cm^3 of soil (Widmer 2014). Subplots were irrigated by hand to wash *Trichoderma* inoculum into the soil. Additional mesh bags from the three depths in each subplot were retrieved 2 and 8 weeks after the biocontrol treatment was applied in California trial 1, and after 4 and 6 weeks in both the California trial 2 and the Oregon field trial.

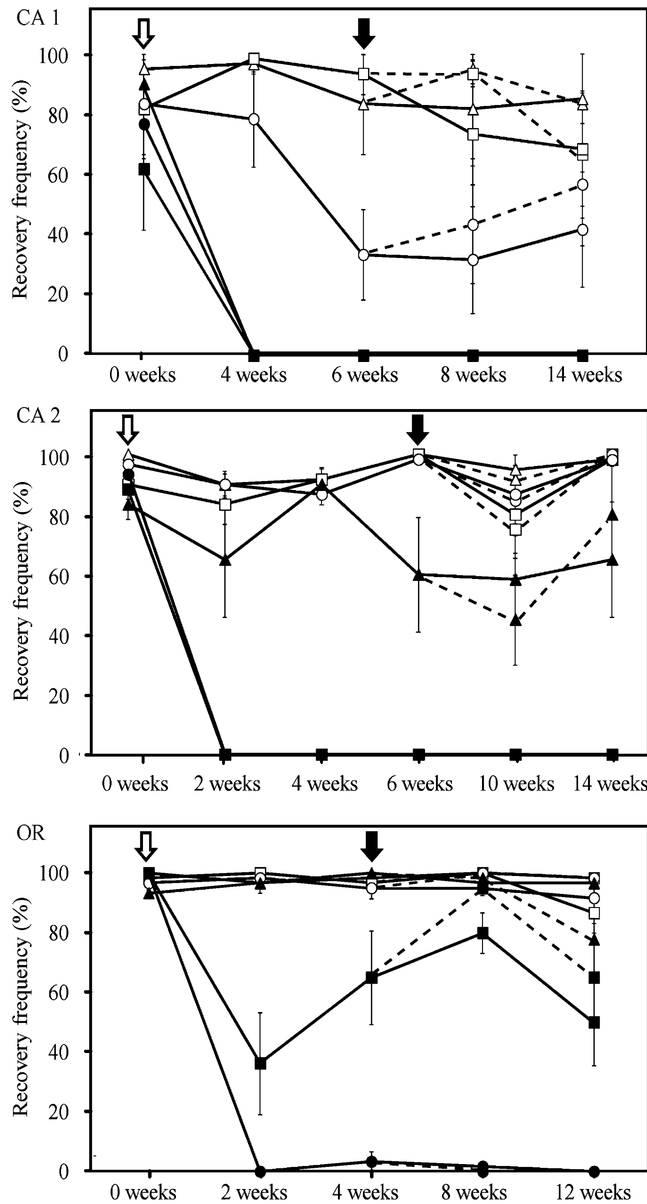

Fig. 2. Frequency of recovery (%) of *Phytophthora* from infested leaf disks buried at soil depths of 5 (○ or ●), 15 (□ or ■), and 30 (△ or ▲) cm during the field trials. Open symbols represent nonsolarized treatments and solid symbols represent solarized treatments. Open arrows indicate the beginning of solarization and solid arrows indicate the time of *Trichoderma asperellum* (TA) application (dashed line = TA and solid line = non-TA) in three field trials (CA 1, CA 2, and OR). Trials CA 1 and CA 2 were conducted with *Phytophthora ramorum* in San Rafael, CA beginning 22 July 2012 or 17 August 2012, respectively. The OR trial was conducted with *P. pini* in Corvallis, OR beginning 24 August 2012.

***P. ramorum* recovery.** All samples were sent to Oregon State University and kept cool (4°C) until processing. Leaf disks were removed \from the mesh bags, rinsed in water to remove soil, and plated on *Phytophthora*-selective medium (PARPH) (Jeffers and Martin 1986) with modified amounts of antibiotics (ampicillin at 200 mg/liter, rifamycin at 10 mg/liter, pentachloronitrobenzene at 66.7 mg/liter, hymexazol at 25 mg/liter, and Delvocid at 20 mg/liter; DSM, Delft, The Netherlands). Plates were examined after 20 days for outgrowth of colonies of *P. ramorum* or *P. pini*, respectively. Recovery was quantified as percentage of leaf disks (out of 10) with outgrowth into the medium.

Quantifying *T. asperellum* soil populations. Soil samples were collected from mesh bags buried at the 5-cm depth for quantification of the *T. asperellum* population. Soil samples (1 g) were suspended in 25 ml of sterile water agar (0.2%) and placed on a shaker for 20 min. A dilution series was prepared (1:500, 1:5000, and 1:50000) and 1 ml of each suspension was spread onto a *Trichoderma*-selective medium (Askew and Laing 1993). Colonies were counted after 1 week of incubation at 20°C. *T. asperellum* colonies were distinguished from other *Trichoderma* spp. by their characteristic colony morphology and verified by sequencing the internal transcribed spacer (ITS) of representative colonies using primers ITS1F and ITS4 (White et al. 1990). Dry weight of soil samples was determined by oven drying soil subsamples and reweighing.

Statistical analysis. The nonparametric Mann-Whitney *U* test was used to compare average mean daily soil temperature of solarized and nonsolarized treatments at each soil depth. The test was used because three replications would not be enough to ascertain a normal distribution. All inoculum recovery data were logit transformed. Analysis of variance (ANOVA) was performed to test the fixed effects of solarization and TA application on the weighted average of logit transformed inoculum recovery. ANOVA was also applied to determine whether the *T. asperellum* population density was significantly different between TA and non-TA treatments, and to determine the effect of solarization treatment on the subsequent establishment of a *T. asperellum* population in TA plots in each trial. The reduced model was derived by stepwise Akaike Information Criterion comparisons before applying ANOVA. All statistical tests were conducted using SPSS Statistics 19.0 software (SPSS Inc., Chicago) at a $P < 0.05$ level of significance.

Results

Soil temperatures. Soil temperatures are summarized for the first 4 weeks in each trial during the solarization period (Table 1). Across all three depths, mean soil temperatures were 8.1 to 9.4, 7.0 to 7.8, and 5.9 to 6.1°C higher in solarized plots than in nonsolarized plots in California trial 1, California trial 2, and the Oregon trial, respectively. Mean soil temperatures in solarized plots in trial 2 in California and the trial in Oregon were 1.9 to 2.6 and 4.8 to 6.1°C lower than those in trial 1 in California across all three depths. The average maximum daily soil temperature at the 5-cm depth in solarized plots achieved 51, 48, and 41°C in trials 1 and 2 in California and the trial in Oregon, respectively. The amplitude of diurnal fluctuation in soil temperature in solarized plots was 18.5 to 24.1°C at the 5-cm depth in contrast to 2.2 to 3.3°C at the 30-cm depth. Also, the amplitude of daily fluctuation was 3.5 to 6.0, 1.6 to 2.9, and 0.6 to 0.9°C greater at depths of 5, 15, and 30 cm, respectively, than in nonsolarized plots. More than 100 h of temperatures greater than 35°C were achieved with 4 weeks of solarization at all three depths in trial 1 in California, at the 5- and 15-cm depths in trial 2 in California, and at the 5-cm depth in the trial in Oregon in solarized plots. Even in nonsolarized plots, 138 and 87 h of temperatures greater than 35°C were achieved with 4 weeks at the 5-cm depth in trial 1 and trial 2 in California, respectively.

Recovery of *Phytophthora* spp. after solarization and TA applications. *P. ramorum* was not recovered from leaf disk inoculum retrieved from depths of 5, 15, and 30 cm in solarized

plots in trial 1 or at 5 and 15 cm in trial 2 in California at the first sampling during solarization in each trial (4 weeks in trial 1 and 2 weeks in trial 2; (Fig. 2; Table 2). *P. ramorum* was recovered from the 30-cm depth in trial 2 in California even after 6 weeks of solarization. *P. ramorum* was recovered from all depths in nonsolarized plots, including 5 cm in California trial 1, where more than 100 h of temperatures greater than 35°C was achieved (Table 1). *P. pini* was not recovered from leaf inoculum retrieved from the 5-cm depth in solarized plots in the trial in Oregon (Fig. 2), except for one sample retrieved after 4 weeks of solarization, in which 2 of 10 leaf disks yielded *P. pini.* The inoculum was always recovered from 15- and 30-cm depths in solarized plots and also from all three depths in nonsolarized plots. TA application did not result in any significant difference in *Phytophthora* spp. recovery for any depth in any trials ($P > 0.05$, ANOVA test; Fig. 2; Table 2).

The enhanced recovery of *P. ramorum* from soil following 30 days of storage at 4°C was reported previously (Tooley and Carras 2011). Therefore, we sought to determine whether *P. ramorum* could be recovered after the winter. We sampled in March 2013 for trial 1 in California, 7 months after solarization, and did not see any significant difference in pathogen recovery when compared with recovery the previous October. We also tested the effect of storage on recovery by storing samples under moist conditions at 4°C and did not observe any significant difference in pathogen recovery. We conclude that the lack of recovery indicated that the pathogen was dead and not simply dormant.

***T. asperellum* population densities.** Application of TA to plots resulted in a population density of *T. asperellum* at the 5-cm depth that was greater than in non-TA plots (Tables 3 and 4; $P < 0.001$, ANOVA). Inconsistent *T. asperellum* population densities with relatively large standard errors (Table 3) were observed among replicates. There was no significant *T. asperellum* establishment observed in non-TA plots ($P = 0.811$, ANOVA). The effect of solarization on *T. asperellum* population density was not significant (Table 4; $P = 0.052$, ANOVA), although population densities were often two- to fourfold greater in solarized plots compared with nonsolarized plots (Table 3). There were a few *T. asperellum* colonies that grew from non-TA-amended soil samples. Because there was no detectable indigenous *T. asperellum* in the soil prior to TA amendment based on plating the field soil, we concluded there was some lateral movement of *T. asperellum.*

Discussion

This study demonstrates that solarization is a potential method for eradicating *P. ramorum* in the surface soil in both California (depths of 0 to 15 or 0 to 30 cm) and Oregon (depths of 0 to 5 cm). Temperatures achieved in solarization trial 1 in San Rafael, CA were 4 to 5°C lower than in previous studies in Davis, CA (Juarez-Palacios et al. 1991; Pullman et al. 1981). The plot size in our study (6.3 m^2) was smaller than the 12 or 36 m^2 used in previous studies (Juarez-Palacios et al. 1991 and Pullman et al. 1981, respectively). Larger plots for solarization likely would have achieved higher soil temperatures by minimizing the border effects (Grinstein et al. 1995). Therefore, the smaller plots in our trials may have underestimated the efficacy of larger-scale solarization in commercial nurseries. Davis, CA also has a 4 to 5°C higher mean air temperature than San Rafael, CA in summer (meteorological data from Remote Automatic Weather Stations; http://raws.fam.nwcg.gov/). Trial 2 in California had a lower temperature because it was carried out later in the season than trial 1. Soil temperature in the Oregon trial was within the range of soil temperature measured in a previous study (Pinkerton et al. 2000). Because 3 to 4°C higher soil temperature was observed in mid-August (Pinkerton et al. 2000) as compared with the period of our field trial, a higher soil temperature in Oregon is likely to have

Table 3. *Trichoderma asperellum* population density (CFU × 10^4 g^{-1} soil) at the 5-cm soil depth after *Trichoderma asperellum* application (TA) following solarization treatments in trials CA 1 and CA 2 in San Rafael, CA and trial OR in Corvallis, OR

			T. asperellum propagule density (CFU × $10^4 g^{-1}$ soil)[a]		
Trial	**Treatments**		**2 weeks**	**4 weeks**	**8 weeks**
CA 1					
	Solarized	TA	3.63 (1.80)	…	2.48 (0.83)
		Non-TA	0.01 (0.01)	…	0.02 (0.02)
	Nonsolarized	TA	1.00 (0.43)	…	0.55 (0.20)
		Non-TA	0.22 (0.18)	…	0.00 (0.00)
CA 2					
	Solarized	TA	…	0.36 (0.15)	1.27 (1.15)
		Non-TA	…	0.00 (0.00)	0.01 (0.01)
	Nonsolarized	TA	…	0.36 (0.31)	0.57 (0.24)
		Non-TA	…	0.00 (0.00)	0.00 (0.00)
OR					
	Solarized	TA	…	1.08 (0.43)	4.20 (0.63)
		Non-TA	…	0.04 (0.03)	0.07 (0.04)
	Nonsolarized	TA	…	2.52 (1.54)	2.31 (0.65)
		Non-TA	…	0.00 (0.00)	0.19 (0.09)

[a] Average (standard error).

Table 2. *P* values of solarization and *Trichoderma asperellum* application (TA) effects from analysis of variance (ANOVA) on recovery (%) of *Phytophthora ramorum* and *P. pini* from infested leaf disks buried at soil depths of 5, 15, and 30 cm in trials CA 1 and CA 2 in San Rafael, CA, and trial OR in Corvallis, OR

					***P* values at depths of**		
Inoculum	**Trial**	**Treatments**[a]	**Time**[b]	**Plot**	**5 cm**	**15 cm**	**30 cm**
P. ramorum	CA 1	Solarization	…	…	0.046	<0.001	<0.001
		TA	2 weeks	Solarized	1.000	1.000	1.000
				Nonsolarized	0.494	0.25	0.844
			8 weeks	Solarized	1.000	1.000	1.000
				Nonsolarized	0.851	0.903	0.671
P. ramorum	CA 2	Solarization	…	…	<0.001	<0.001	0.835
		TA	4 weeks	Solarized	1.000	1.000	0.969
				Nonsolarized	0.999	0.731	0.877
			8 weeks	Solarized	1.000	1.000	0.38
				Nonsolarized	0.146	0.154	0.154
P. pini	OR	Solarization	…	…	<0.001	0.25	0.075
		TA	4 weeks	Solarized	0.154	0.093	0.486
				Nonsolarized	0.012	1.000	1.000
			8 weeks	Solarized	1.000	0.823	0.148
				Nonsolarized	0.114	0.033	1.000

[a] For solarization, *P* values are shown associated with Day × Solarization from ANOVA based on the model of logit(Recovery) = Intercept + Day + Solarization + Day × Solarization ($n = 6$). For TA, *P* values are shown associated with TA from ANOVA based on the model of logit(Recovery) = Intercept + TA ($n = 6$).
[b] Time since *T. asperellum* application.

been achieved in our study if the solarization had been conducted in July or earlier in August.

P. pini was not recovered from at least the upper 5-cm depth in Oregon in solarized plots (Fig. 1). Because *P. pini* is more heat tolerant than *P. ramorum* (Jung and Burgess 2009; Werres et al. 2001), it is not likely that *P. ramorum* would have survived where *P. pini* was not recovered. Because *P. ramorum* cannot be tested outside of quarantine facilities, *P. pini* can be a good indicator of solarization efficacy. The low effectiveness of solarization in deeper horizons in Oregon could result from either the lower temperature or the higher thermal tolerance of *P. pini* compared with *P. ramorum*. Direct comparison between *P. ramorum* and *P. pini* in the same location would help interpret the usefulness of *P. pini* as a surrogate for *P. ramorum* in field studies.

Cumulative hours of solarization in the surface layer of soils appeared to exceed the threshold required to kill the pathogens. The reported length required to kill *P. ramorum* is 96 h at 35°C or 48 h at 40°C (Tooley et al. 2008). Therefore, it may be possible to shorten the period of solarization, making it more feasible for growers to apply solarization to nurseries. Solarized strawberry or raspberry fields in Washington were also reported to have had more than 96 cumulative hours above 35°C in the surface 0 to 10 cm with 8 weeks of solarization (Pinkerton et al. 2002, 2009). These data indicate the possible use of solarization to eradicate *P. ramorum* in surface soil in Washington nurseries as well.

A study in three retail nurseries in Washington in which the soil had previously tested positive for *P. ramorum* showed that *P. ramorum* was not limited to the organic layer of nursery soil but was detected in the top 0-to-10-cm soil layer (Dart et al. 2007). Solarization may not be able to eradicate pathogens at deeper horizons in cooler locations. Because the field surface in container nurseries tends to be covered by crushed rock to facilitate drainage, further tests are required to determine the effect of the rock layer on the elevated soil temperature by solarization. *P. ramorum* was still recovered from the 5-cm depth in nonsolarized plots in trial 1 in California even though the cumulative hours exposed to high soil temperature should have been adequate to kill the pathogen. During the 4-week trial, the inoculum at the 5-cm depth was exposed to 138 h above 35°C and 48 h above 40°C. In contrast, *P. ramorum* was not recovered at the 30-cm depth in solarized plots, where the inoculum only experienced 130 h above 35°C and 0 h above 40°C. This indicates that the pathogen response to heat is most likely not cumulative and may involve other factors. In nonsolarized plots, surface soil was drier than in solarized plots due to evaporation (data not shown). Dry conditions have been reported to increase the tolerance of pathogens to heat, as observed with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Shlevin et al. 2004), *Alternaria porri* f. sp. *solani* (Rotem 1968), and *Sclerotium oryzae* (Usmani and Ghaffar 1986). Diurnal fluctuations are large at the surface horizons, resulting from greater maximum and lower minimum temperatures, and leading to the intermittent exposure of the pathogen to heat. Continuous exposure to heat had a greater effect than intermittent exposure on *S. sclerotiorum* in soil (Porter 1991). Consideration of soil moisture and intermittent heat may more precisely predict pathogen responses to solarization.

Table 4. Analysis of variance (ANOVA) of *Trichoderma asperellum* population density (CFU × 10^4/g soil) in relation to *T. asperellum* application (TA), trial, and solarization treatment[a]

Effect[b]	df	Sum Sq	Mean Sq	*F* value	*P* value
TA	1	93.2	93.2	37.8	<0.001
Trial	2	20.7	10.4	4.2	0.017
TA × trial	2	17.1	8.6	3.5	0.034
TA × solarization	2	14.9	7.5	3.0	0.052
Residuals	128	315.6	2.5	…	…

[a] All soil samples were from the 5-cm depth; df = degrees of freedom, Sum Sq = sum of squares, and Mean Sq = mean squares. The reduced model was determined by stepwise Akaike Information Criterion (AIC) comparisons to be Population = Intercept + TA + Trial + TA × Trial + TA × Solarization.

[b] TA = *T. asperellum* application or no application; Trial = trials 1 and 2 in San Rafael, CA and in Corvallis, OR; and Solarization = solarized or nonsolarized treatment.

Our results did not show a strong effect of solarization on establishment of the *T. asperellum* biocontrol agent even though there was a moderate increase in *T. asperellum* population in solarized plots compared with nonsolarized plots. Solarization was expected to enhance establishment of the introduced biocontrol agent by reducing competition by other soil microorganisms (Jayaraj and Radhakrishnan 2008). Our results contrast with the reduced *P. ramorum* populations in TA-amended plots associated with the increased *T. asperellum* population over time observed by Widmer et al. (2011). One reason may be that the *T. asperellum* population density in the field soils was not high enough to be effective. Preliminary studies showed that *T. asperellum* concentration of 1×10^7 CFU/cm^3 soil is necessary to effectively eliminate *P. ramorum* (T. L. Widmer, unpublished data). Infiltration of biocontrol inoculum into the soil by watering may not be effective in soils with a high bulk density, as with our soil (Van Elsas et al. 1991). Another reason may be the type of inoculum used. In this study, we used infected leaf inoculum instead of "naked" chlamydospores, as used by Widmer et al. (2011). *T. asperellum* is not known to colonize leaf material; therefore, inoculum of the pathogen inside leaves may have been protected from parasitism. A single surviving *Phytophthora* chlamydospore is expected to result in colony outgrowth from the leaf disk in our evaluation of survival. Although *T. asperellum* might have killed a portion of the pathogen population in the leaf disks, our experiment was not designed to detect the decline of pathogen population within each leaf disk. Nevertheless, a potential benefit of using *T. asperellum* would be to prevent reinfestation of the previously disinfested soil by creating soil suppressiveness; this aspect would require future study.

This study showed that soil solarization has an excellent potential for disinfesting surface soil in container nurseries. *P. pini* could be used as a surrogate for *P. ramorum* to test the efficacy of solarization outside of quarantine facilities. Soil solarization also has the potential to control other pathogens that might be present in the soil. On the other hand, *T. asperellum* did not appear to reduce recovery of the pathogens in our system. Further studies are necessary to determine the effect of different water potentials and diurnal temperature fluctuation on the response of pathogens to heat and to test how a layer of crushed rock on the soil surface affects solarization efficacy. Additional research is also needed to test solarization on other soilborne *Phytophthora* spp. common in container nurseries (Parke et al. 2014).

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Literature Cited

Affokpon, A., Coyne, D. L., Htay, C. C., Agbede, R. D., Lawouin, L., and Coosemans, J. 2011. Biocontrol potential of native *Trichoderma* isolates against root-knot nematodes in West African vegetable production systems. Soil Biol. Biochem. 43:600-608.

Askew, D. J., and Laing, M. D. 1993. An adapted selective medium for the quantitative isolation of *Trichoderma* species. Plant Pathol. 42:686-690.

Benítez, T., Rincón, A. M., Limón, M. C., and Codón, A. C. 2004. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7:249-260.

Dart, N. L., Chastagner, G. A., Rugarber, E. F., and Riley, K. L. 2007. Recovery frequency of *Phytophthora ramorum* and other *Phytophthora* spp. in the soil profile of ornamental retail nurseries. Plant Dis. 91:1419-1422.

Gamliel, A., and Katan, J. 2012. Soil solarization: Theory and Practice. American Phytopathological Society, St. Paul, MN.

Goss, E. M., Larsen, M., Chastagner, G. A., Givens, D. R., and Grünwald, N. J. 2009. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS Pathog. 5(9):e1000583.

Grinstein, A., Kritzman, G., Hetzroni, A., Gamliel, A., Mor, M., and Katan, J. 1995. The border effect of soil solarization. Crop Prot. 14:315-320.

Harman, G. E. 2000. Myths and dogmas of biocontrol—Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis. 84:377-393.

Harman, G. E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 96:190-194.

Jayaraj, J., and Radhakrishnan, N. V. 2008. Enhanced activity of introduced biocontrol agents in solarized soils and its implications on the integrated control of tomato damping-off caused by *Pythium* spp. Plant Soil 304:189-197.

Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.

Juarez-Palacios, C., Felix-Gastelum, R., Wakeman, R. J., Paplomatas, E. J., and Devay, J. E. 1991. Thermal sensitivity of three species of *Phytophthora* and the effect of soil solarization on their survival. Plant Dis. 75:1160-1164.

Jung, T., and Burgess, T. I. 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. Persoonia 22:95-110.

Kelley, W. D., and Rodriguez-Kabana, R. 1976. Competition between *Phytophthora cinnamomi* and *Trichoderma* spp. in autoclaved soil. Can. J. Microbiol. 22:1120-1127.

Linderman, R. G., and Davis, E. A. 2008. Eradication of *Phytophthora ramorum* and other pathogens from potting medium or soil by treatment with aerated steam or fumigation with metam sodium. HortTechnology 18:106-110.

Ochiai, N., Dragiila, M. I., and Parke, J. L. 2011. Pattern swimming of *Phytophthora citricola* zoospores: An example of microbial bioconvection. Fungal Biol. 115:228-235.

Parke, J. L., Knaus, B. J., Fieland, V. J., Lewis, C., and Grünwald, N. J. 2014. *Phytophthora* community structure analyses in Oregon nurseries inform systems approaches to disease management. Phytopathology 104:1052-1062.

Parke, J. L., and Lewis, C. 2007. Root and stem infection of rhododendron from potting medium infested with *Phytophthora ramorum*. Plant Dis. 91:1265-1270.

Pinkerton, J. N., Bristow, P. R., Windom, G. E., and Walters, T. W. 2009. Soil solarization as a component of an integrated program for control of raspberry root rot. Plant Dis. 93:452-458.

Pinkerton, J. N., Ivors, K. L., Miller, M. L., and Moore, L. W. 2000. Effect of soil solarization and cover crops on populations of selected soilborne plant pathogens in western Oregon. Plant Dis. 84:952-960.

Pinkerton, J. N., Ivors, K. L., Reeser, P. W., Bristow, P. R., and Windom, G. E. 2002. The use of soil solarization for the management of soilborne plant pathogens in strawberry and red raspberry production. Plant Dis. 86:645-651.

Porras, M., Barran, C., Arroyo, F. T., Santos, B., Blanco, C., and Romero, F. 2007. Reduction of *Phytophthora cactorum* in strawberry fields by *Trichoderma* spp. and soil solarization. Plant Dis. 91:142-146.

Porter, I. J. 1991. Factors which influence the effectiveness of solarization for control of soilborne fungal pathogens in South Eastern Australia. Ph.D. dissertation, La Trobe University, Bundoora, Victoria, Australia.

Pullman, G. S., Devay, J. E., Garber, R. H., and Weinhold, A. R. 1981. Soil solarization—Effects on Verticillium wilt of cotton and soilborne populations of *Verticillium dahliae, Pythium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola*. Phytopathology 71:954-959.

Rotem, J. 1968. Thermoxerophytic properties of *Alternaria porri* f. sp. *solani*. Phytopathology 58:1284-1287.

Shishkoff, N. 2007. Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. Plant Dis. 91:1245-1249.

Shlevin, E., Mahrer, Y., and Katan, J. 2004. Effect of moisture on thermal inactivation of soilborne pathogens under structural solarization. Phytopathology 94:132-137.

Smith, V. L., Wilcox, W. F., and Harman, G. E. 1990. Potential for biological-control of *Phytophthora* root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathology 80:880-885.

Tooley, P. W., Browning, M., and Berner, D. 2008. Recovery of *Phytophthora ramorum* following exposure to temperature extremes. Plant Dis. 92:431-437.

Tooley, P. W., and Carras, M. M. 2011. Enhanced recovery of *Phytophthora ramorum* from soil following 30 days of storage at 4 degrees C. J. Phytopathol. 159:641-643.

Usmani, S. M. H., and Ghaffar, A. 1986. Time temperature relationships for the inactivation of sclerotia of *Sclerotium oryzae*. Soil Biol. Biochem. 18: 493-496.

Van Elsas, J. D., Trevors, J. T., and Vanoverbeek, L. S. 1991. Influence of soil properties on the vertical movement of genetically-marked *Pseudomonas fluorescens* through large soil microcosms. Biol. Fertil. Soils 10:249-255.

Vercauteren, A., Riedel, M., Maes, M., Werres, S., and Heungens, K. 2012. Survival of *Phytophthora ramorum* in rhododendron root balls and in rootless substrates. Plant Pathol. 62:166-176.

Washington, W. S., Engleitner, S., Boontjes, G., and Shanmuganathan, N. 1999. Effect of fungicides, seaweed extracts, tea tree oil, and fungal agents on fruit rot and yield in strawberry. Aust. J. Exp. Agric. 39:487-494.

Werres, S., Marwitz, R., Veld, W., De Cock, A., Bonants, P. J. M., De Weerdt, M., Themann, K., Ilieva, E., and Baayen, R. P. 2001. *Phytophthora ramorum* sp. nov., a new pathogen on Rhododendron and Viburnum. Mycol. Res. 105: 1155-1165.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.

Widmer, T. L. 2014. Screening *Trichoderma* species for biological control activity against *Phytophthora ramorum* in soil. Biol. Control 79:43-48.

Widmer, T. L., Johnson-Brousseau, S. A., and Ghosh, S. 2011. Management of *Phytophthora ramorum*-infested nursery soil with *Trichoderma asperellum*. (Abstr.) Phytopathology 101:S191.

Yakabe, L. E., and MacDonald, J. D. 2010. Soil treatments for the potential elimination of *Phytophthora ramorum* in ornamental nursery beds. Plant Dis. 94:320-324.