Soil Solarization to Eradicate Soilborne *Phytophthora* spp. in Container Nurseries with Surface Gravel[1]

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Abstract

To describe the effect of soil solarization in the presence of a gravel layer on the soil surface of container nurseries, we investigated belowground temperatures and soil water potential during solarization with different thicknesses of a surface gravel layer (2.5 cm, 7.5 cm, or no gravel) (1 in, 3 in, or no gravel) in relation to survival of soilborne *Phytophthora* spp. inoculum. In field trials conducted for 4 weeks with *Phytophthora ramorum* Werres and *Phytophthora pini* Leonian in San Rafael, California and with *P. pini* in Corvallis, Oregon, infested rhododendron leaf inoculum was placed on the surface, and at 5 cm (2 in) and 15 cm (6 in) below the surface. In solarized plots with thicker layers of gravel, inoculum buried in the soil layer was killed in shorter treatment periods by higher elevated temperatures. Inoculum at the surface and within the gravel layer was also killed, but showed greater tolerance to heat under the lower water potential conditions as compared to the soil layer. *P. pini* has a significantly longer survival in heat than *P. ramorum,* allowing it to serve as a conservative surrogate for *P. ramorum* in testing solarization outside the quarantine facility. This study demonstrates how presence of a gravel layer influences soil solarization effectiveness in reducing *Phytophthora* inoculum survival.

Index words: *Phytophthora ramorum*, *Phytophthora pini*, soil disinfestation, disease management, soil temperature, soil water potential, ornamentals.

Species used in this study: *Phytophthora ramorum* Werres, de Cock & Man in't Veld, *Phytophthora pini* Leonian.

Significance to the Horticulture Industry

Soil solarization is expected to provide effective management of many soilborne diseases, including ones caused by *Phytophthora* spp. in nurseries. However, container nursery beds are often covered by a layer of gravel, and the effect of the gravel layer on soil solarization efficacy has not been described. This research provides critical information to nursery managers about the effect of different thicknesses of gravel on belowground temperature and moisture conditions during soil solarization. Our findings demonstrate that presence of a gravel layer can be expected to increase the belowground temperature during soil solarization, but also indicate the potential for extended survival of the inoculum in the gravel layer by being in a dry condition. Our results suggest that the 2.5 cm gravel treatment would be sufficient to enhance the effect of soil solarization throughout the soil profile, at the same time minimizing the thickness of the drier layer where the inoculum becomes more tolerant to heat. Although solarization is a promising method for eradicating *Phytophthora ramorum*, causal agent of sudden oak death (SOD), it is important to expand the research to include another species of *Phytophthora* as a possible proxy for *P. ramorum* in evaluating solarization efficacy in other locations because *P. ramorum* cannot be tested outside a quarantine facility. This research also provides a direct comparison between *P. ramorum* and *P. pini* in evaluating solarization efficacy and suggests that *P. pini* is a useful indicator of lethal conditions of solarization for *P. ramorum*.

Introduction

Container nursery beds are often covered by a layer of gravel to improve surface drainage and to reduce the likelihood of plant disease resulting from direct contact of containers with native soil. A 7.5 cm (3 in) layer of gravel layer on top of nursery beds is generally recommended (Griesbach et al. 2012), although many nurseries use only about 2.5 cm (1 in) of gravel to reduce costs.

Soil solarization is expected to provide effective management of many soilborne diseases (Gamliel and Katan 2012), and it is a practical method for container nurseries as well (Funahashi and Parke 2016). However, most reports about solarization concern agricultural fields in bare soil, and there are no studies conducted in soils covered with gravel. A few studies reported a gravel-sand mulch effect on soil temperature and moisture conditions in a few crop systems (Lu et al. 2013, Li 2003, Nachtergaele et al. 1998). Some of the treatments were combined with plastic mulch to increase soil temperature and to improve water storage (Wang et al. 2011). However, none of this research focused on solarization for control of soilborne disease.

The quarantine pathogen *Phytophthora ramorum,* causal agent of sudden oak death (SOD), can be spread by movement of infected nursery plants (Goss et al. 2009). There were a total of 612 nursery detections in the U.S. between 2001 and 2018, and more than 250 detections in

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14 states in 2019 (COMTF 2020, USDA-APHIS 2015). Although *P. ramorum* is recognized as a pathogen of stems and leaves, it also infects roots of nursery plants (Parke and Lewis 2007, Shishkoff 2007) and can infest potting media and soil, where it can survive in the absence of a host for at least 33 months (Vercauteren et al. 2013). Soilborne inoculum in nursery beds has been detected from the top 0 to 10 cm (0 to 4 in) (Dart et al. 2007), likely contributing to the persistence of the pathogen in nurseries from year to year. *P. ramorum* chlamydospores, the main survival structure for this species, were killed by exposure to 30 min of 50 C (122 F) (Linderman and Davis 2008), 2 days of 40 C (104 F), and 4 days of 35 C (95 F) (Tooley et al. 2008). Our previous field study showed that soil solarization is a promising method for eradicating soilborne *Phytophthora* inoculum (Funahashi and Parke 2016). However, the study was conducted on bare soil, and the effect of surface gravel, typical of container nurseries, on solarization efficacy was not tested. Because *P. ramorum* cannot be tested outside a quarantine facility, limiting the geographic scope of the study, it was important to expand the research to include another species of *Phytophthora* to test as a possible proxy for *P. ramorum* in evaluating solarization efficacy in other locations. *P. pini* Leonian (previously *P. citricola* Sawada) is another destructive plant pathogen in container nurseries. Although it tolerates higher temperatures than *P. ramorum* (Werres et al. 2001, Jung and Burgess 2009, Funahashi and Parke 2018), we selected it to enable direct comparison between *P. ramorum* and *P. pini* in evaluating solarization efficacy. The objectives of this study were 1) to investigate the effect of different thicknesses of gravel on belowground temperature and moisture conditions during soil solarization, and 2) to determine how these different gravel thicknesses affected survival of soilborne *P. ramorum* and *P. pini* inoculum.

Materials and Methods

Field experiments. Three field trials with a similar experimental design were conducted to test the effect of surface gravel thickness on solarization efficacy and survival of *Phytophthora* inoculum. Two sites were utilized: the National Ornamentals Research Site at Dominican University of California, San Rafael, California (CA), and the Oregon State University Botany Farm and Field Lab, Corvallis, Oregon (OR). Two trials were conducted in CA. Trial 1 began July 16, 2013 with *P. ramorum* and trial 2 began August 24, 2013 with both *P. ramorum* and *P. pini*. Another trial was carried out with *P. pini* in OR beginning August 2, 2013. There was no precipitation recorded during the trials.

The soil at the CA site was a sandy loam "fill" (sand 55.2%, silt 25.2%, clay 19.6%; gravel fraction 27.0%, bulk density 1.85 g·cm^{-3}, pH 6.4, organic matter 4.7%). Soil in OR 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, organic matter 2.6%). Gravel fraction is the composition of particles larger than 2 mm.

Each trial consisted of 18 plots except for trial 2 that had 24 plots, each 2.5 m by 2.5 m (8.2 by 8.2 ft), and treatments were arranged in a randomized factorial block design with three replications per solarization (solarized or non-solarized) and gravel layer thickness treatment (0, 2.5, 7.5 cm). The gravel layer thicknesses were chosen to simulate the range of conditions typical of commercial container nurseries. Plots were left bare, or gravel [(average diameter approximately 1.9 cm (3/4 in)] was applied to the surface. Solarized treatment plots were then covered with a transparent (clear) plastic sheet and non-solarized treatment plots were left uncovered. In trial 2, 6 plots with *P. pini,* (3 solarized, 3 non-solarized), each with a 2.5-cm-thick gravel layer, were added for comparison with *P. ramorum*.

Phytophthora *spp. inoculum. P. ramorum* Werres, de Cock & Man in't Veld isolate Pr-1418886 was used in CA and *P. pini* Leonian isolate Pc98-517 was used in OR to produce infested leaf disk inoculum. *P. ramorum* zoospores were produced according to established methods (Parke and Lewis 2007) but with dilute (1/3 strength) V8 broth agar substituted for V8-CMA. *P. pini* zoospores were produced according to Ochiai et al. (2011). *Rhododendron catawbiense* Michx. '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 per mL) and incubated in a moist chamber at 20 C (*P. ramorum*) or 24 C (*P. pini*). After 2 weeks, 6-mm-diam disks were removed from leaf lesions using a hole punch. The presence of chlamydospores (*P. ramorum*) or oospores (*P. pini*) within leaf tissue prior to use as inoculum was confirmed by microscopic observation of cleared leaves processed as described in Philips and Hayman (1970). Mesh bags (4 cm by 4 cm) were constructed from nylon phytoplankton netting (100-µm opening) (Aquatic Ecosystems, Apopka, FL) and 10 infested leaf disks were placed inside. Another set of mesh bags ,each containing 20 leaf disks, was also prepared for determination of leaf water content at the interface between the soil surface and the gravel layer. Eight columns (8 cm diam by 20 cm depth) were prepared for each plot. Columns were constructed with two inner columns of different length (10 cm and 20 cm) made from plastic window screen material to allow for drainage and aeration. The shorter one was stacked on top of the longer one to allow sampling at different times with minimal disturbance (Fig. 1). The columns were filled with field soil or gravel consistent with the surrounding substrate treatment, and mesh bags with leaf disk inoculum were inserted at 0 cm, 5 cm, and 15 cm depths below the soil or gravel surface. Columns were placed in cylindrical holes (12 cm diameter by 25 cm deep) arranged in a circular pattern 40 cm from the center of each plot and filled with field soil or gravel (Funahashi and Parke 2016). A temperature probe (CS109-L, Campbell Scientific, Logan, UT) was placed at 0 cm in the center of each plot and at the 5 cm depth for the 7.5 cm gravel treatment plots. Soil water content reflectometers (CS655, Campbell Scientific, Logan, UT) were placed at 5 cm and 15 cm depths for 0 cm or 2.5 cm gravel treatment plots and at 15 cm for the 7.5 cm gravel treatment plots. Soil temperature and volumetric water content (VWC) data were recorded every 30 min with a CR1000 datalogger

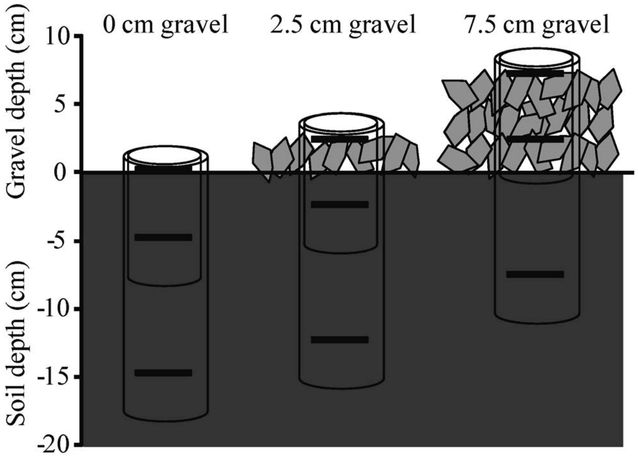

Fig. 1. Schematic diagram of the gravel treatments in the field trials and columns with mesh bag samples (closed horizontal bars) containing infested leaf disks. Cylindrical holes each held two columns of different lengths (a 10 cm inner column and a 20 cm outer column) to allow sampling at different times with minimal disturbance. The columns were filled with field soil or gravel consistent with the surrounding substrate treatment, and mesh bags with leaf disk inoculum were inserted at 0 cm, 5 cm, and 15 cm depths below the soil or gravel surface.

with an AM16/32B relay multiplexer (Campbell Scientific, Logan, UT). We used these sensors to monitor temperature at the same depth as the leaf disc inoculum and to monitor volumetric water content for ones in soil (Fig. 1).

Field sites were irrigated to saturation, allowed to drain overnight, and initial inoculum samples were collected the next day. Then, plots in the solarization treatment were each covered by a transparent 0.15 mm thick (6-mil) anti-condensation 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 non-solarized plots were left uncovered. Solarization was conducted for 4 weeks. Different schedules consisting of three sampling times were planned for each treatment to capture the timing of maximum inoculum survival. Two mesh bags from each plot were retrieved at each sampling time. During the solarization period, samples from the solarized treatment were collected by making small cuts in the plastic sheets, retrieving samples, and sealing the cuts with clear tape. There was a minimal effect on soil temperature and moisture.

Phytophthora 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 (200 $mg \cdot L^{-1}$ ampicillin, 10 $mg \cdot L^{-1}$ rifamycin, 66.7 $mg \cdot L^{-1}$ PCNB, 25 $mg \cdot L^{-1}$ hymexazol, and 20 $mg \cdot L^{-1}$ Delvocid, DSM; Delft, The Netherlands). Plates were examined after 14 days for outgrowth of colonies of *P. ramorum* or *P. pini*, respectively. Recovery was quantified as the percentage of leaf disks (out of 10) with outgrowth into the medium.

Water potential of leaf disks at the surface or in the gravel layer. The mesh bags to be used to quantify leaf water content were retrieved at the same time as inoculum recovery samples. Fresh and dry weight (after oven drying for 2 days at 60 C) of leaf disks were measured and water content was calculated. The pressure-moisture curve was established in the lab with a sample of the same leaf disk inoculum using a dew-point potentiometer (WP4, Decagon Devices, Pullman, WA) (Nardini et al. 2008), and water potential of the field samples was calculated from volumetric soil moisture data.

Soil pressure-moisture curve. Water content of the soil at water potential values of -0.034, -0.069, -0.207, -0.414 MPa was measured with a pressure plate apparatus (Soil Moisture Equipment Corporation, Santa Barbara, CA) using the methods of Klute (1986). Water potential of samples dryer than -0.5 MPa was measured with a WP4 dew-point potentiometer. Water retention curves were fitted to a van Genuchten retention curve model (Van Genuchten et al. 1991) using RETention Curve (RETC) and soil hydraulic parameters were derived. Water retention curves were used for calculating soil water potential in field trials using VWC data.

Statistical analysis. The daily temperature regime was very consistent over time with continuous sunny days during all trials. Average daily mean temperature (T_{ave}), average daily maximum temperature (T_{max}), and average daily minimum temperature (T_{min}) were calculated over four weeks for each treatment of each trial. Van Wijk (1963) described daily soil temperature using the equation:

$$T(z,t) = T_{ave} + A_0[\sin(\omega t - z/d)]/\exp(z/d) \qquad \text{(eq. 1)}$$

where $T(z,t)$ is soil temperature at a particular time of day, t, at soil depth, z, T_{ave} is the average temperature of the surface as well as of the profile, A_0 is the amplitude of the surface temperature fluctuation (the range from maximum, or from minimum, to the average temperature). ω is the radial frequency, and d is a characteristic depth, called the damping depth, at which the temperature amplitude decreases to the fraction 1/e of A_0. We first confirmed that average daily temperature was similar at all depths using stepwise Akaike Information Criterion (AIC) comparisons and analysis of variance (ANOVA). Significant factors in the most reduced model were solarization, trial, and interaction terms (solarization×gravel, trial×gravel, and solarization×trial×gravel). The addition of depth to the model did not significantly reduce AIC ($P = 0.687$, ANOVA). We noticed that the upper amplitude (T_{max}-T_{ave}) and lower amplitude (T_{ave}-T_{min}) differed significantly. Therefore, we used the model below to statistically assess the effect of treatments on T_{max} or T_{min}:

$$T_{max.or.min} = T_{ave} + \left(A_{upper} \times dummy1 - A_{lower} \times dummy2\right)/\exp(z/d) \qquad \text{(eq. 2)}$$

where A_{upper} is the upper amplitude at the surface, A_{lower} is the lower amplitude, *dummy1* value is 1 for T_{max} and 0 for T_{min} calculation, and *dummy2* value is 0 for T_{max} and 1 for

Table 1. Estimates of each parameter (± std. error) in the average maximum and minimum daily temperature model (equation 2) and in the volumetric water content exponential decay model (equation 3) for each treatment.

Trial	Gravel (cm)	Solarization	Parameters[z]											
			T_{ave} (C)		A_{upper} (C)		A_{lower} (C)		d (cm)		λ*100 (5 cm)		λ*100 (15 cm)	
CA1	0	Non-solarized	27.7	(1.2)	16.1	(4.7)	14.5	(4.2)	11.1	(1.8)	7.97	(0.14)	2.28	(0.03)
	2.5	Non-solarized	28.4	(1.3)	12.7	(4.7)	15.5	(5.8)	9.1	(1.5)	6.28	(0.07)	4.47	(0.05)
	7.5	Non-solarized	25.2	(0.9)	18.4	(2.6)	11.2	(1.6)	10.4	(1.7)	-	-	4.78	(0.06)
	0	Solarized	31.7	(0.4)	24.3	(1.2)	7.9	(0.4)	13.1	(0.7)	2.40	(0.03)	2.45	(0.03)
	2.5	Solarized	33.6	(1.2)	26.5	(4.6)	7.3	(1.3)	13.6	(3.9)	1.42	(0.02)	2.01	(0.03)
	7.5	Solarized	34.0	(0.4)	34.4	(2.7)	11.2	(0.9)	11.9	(0.6)	-	-	2.33	(0.04)
CA2	0	Non-solarized	27.0	(1.1)	15.9	(2.7)	11.5	(1.9)	10.7	(2.0)	5.45	(0.08)	4.44	(0.05)
	2.5	Non-solarized	26.3	(1.0)	16.3	(2.2)	13.0	(1.8)	10.1	(1.5)	5.41	(0.05)	4.10	(0.04)
	7.5	Non-solarized	26.0	(0.5)	18.1	(1.7)	12.6	(1.2)	10.5	(0.8)	-	-	3.55	(0.05)
	0	Solarized	31.5	(0.1)	20.7	(0.2)	8.3	(0.1)	14.4	(0.2)	3.52	(0.07)	1.28	(0.02)
	2.5	Solarized	34.5	(1.5)	22.2	(4.6)	10.9	(2.2)	12.5	(2.2)	1.98	(0.03)	2.48	(0.03)
	7.5	Solarized	33.2	(1.4)	28.2	(4.6)	9.8	(1.6)	12.0	(1.6)	-	-	2.84	(0.04)
OR	0	Non-solarized	25.9	(1.4)	18.7	(8.6)	10.9	(5.0)	10.0	(2.6)	8.55	(0.14)	4.99	(0.07)
	2.5	Non-solarized	25.5	(1.3)	15.0	(4.1)	13.0	(3.6)	7.9	(1.4)	10.59	(0.19)	7.35	(0.12)
	7.5	Non-solarized	24.1	(0.3)	19.4	(1.7)	11.8	(1.0)	8.6	(0.4)	-	-	5.82	(0.09)
	0	Solarized	31.5	(0.2)	22.8	(0.6)	10.0	(0.2)	12.6	(0.4)	3.37	(0.05)	5.80	(0.09)
	2.5	Solarized	33.8	(0.9)	22.9	(4.8)	9.9	(2.1)	13.1	(1.8)	3.92	(0.06)	2.95	(0.04)
	7.5	Solarized	34.0	(0.5)	28.7	(1.9)	13.8	(0.9)	9.9	(0.5)	-	-	4.13	(0.06)

[z]T_{ave}: Average daily mean temperature (C), A_{upper}: upper amplitude, A_{lower}: lower amplitude, d: damping depth in equation 2 and λ: proportionality constant at 5 cm or 15 cm depth in equation 3.

T_{min} calculation. Each coefficient was compared using a general linear model with factors of trial, solarization, and gravel treatment. The reduced model was achieved using stepwise AIC comparison and ANOVA, and significant factors were investigated for each coefficient. The verification of estimated coefficients calculated from each reduced model was tested by comparing calculated T_{max} and T_{min} for each treatment by the model and observed values.

Decline in the volumetric water content over four weeks was analyzed by fitting to the exponential decay model (Hillel 1998) using the equation below:

$$W(t) = W_i / \exp(\lambda \times t) \quad \text{(eq. 3)}$$

where $W(t)$ is the water content at time t, W_i is the initial water content, and λ is a proportionality constant that can be considered a moisture loss time constant for a specific soil texture. In this study λ is used to quantify the effect of various treatments on the moisture loss process. Measured saturated water contents were used for W_i for each soil ($W_i = 0.414$ for CA and 0.403 for OR). The effects of trial, solarization, depth, and gravel treatment on constant λ were tested by using a general linear model on samples from 0 and 2.5 cm gravel treatment plots. Similarly, the effects of trial, solarization, and gravel treatment on constant λ were tested on samples at 15 cm depth from all plots. The reduced model was achieved using stepwise AIC comparisons and ANOVA, and significant factors were investigated.

ANOVA was also applied to evaluate the effect of gravel thickness on the water potential of leaf inoculum at the surface as well as to evaluate the effect of depth by using samples from the 7.5 cm gravel treatment plots. All inoculum recovery data were logit transformed. ANOVA was performed to test the fixed effects of solarization, gravel thickness, and inoculum type on weighted average of logit transformed inoculum recovery for each depth of each trial. All statistical tests were conducted using R statistics software version 3.1.2 (R Development Core Team, 2014) at a $P < 0.05$ level of significance.

Results and Discussion

Depth did not significantly affect the average daily temperature ($P = 0.687$, ANOVA). Therefore, the parameter T_{ave} was used in equation 2 as the average daily temperature of the profile. The average maximum and minimum daily temperature model (equation 2) fit all data with small standard errors, and each parameter was successfully estimated for each treatment (Table 1). The reduced model was achieved for each parameter with significant factors of either trial, solarization, and/or gravel treatment (Table 2), and coefficients in the reduced model were determined. T_{max} and T_{min} calculated by equation 2 with the parameters estimated from the reduced models strongly correlated with observed values with a high coefficient of correlation (Fig. 2). This shows that the derived reduced models for parameters in statistical analysis successfully explained the significant treatment effects.

T_{ave} was 3.9 to 9.8 C higher ($P < 0.001$) in solarized plots than in non-solarized plots (Fig. 3, Tables 1 and 2). The amplitude ($A_{upper} + A_{lower}$) was also significantly greater (1.5 to 16.0 C) in solarized plots than in non-solarized plots (Fig. 3, Table 1 and 2). The damping depth, d, was 1.4 to 5.2 cm greater in solarized plots than in non-solarized plots ($P < 0.001$). The significantly higher average temperature in solarized plots is consistent with our previous study (Funahashi and Parke 2016) and other field studies (Gamliel and Katan 2012). The greater daily amplitude in solarized plots than in non-solarized plots is also consistent with previous studies (e.g. Pinkerton et al. 2000, Pinkerton et al. 2009, Nyczepir et al. 2012, Peachey et al. 2001). The greater damping depth, d, in solarized

Table 2. ANOVA table of the reduced model to estimate the parameters (T_{ave}, A_{upper}, A_{diff}, and d) in average maximum and minimum temperature model (equation 2). AIC and ANOVA comparisons were used to achieve the reduced model for each parameter.

Parameter[z]	Effect[y]	Df	Sum Sq	Mean Sq	*F* value	*P* value	
T_{ave}	Trial	2	131.0	65.5	44.0	< 0.001	***
	Solarization	1	785.9	785.9	528.5	< 0.001	***
	Gravel	1	13.2	13.2	8.9	0.012	*
	Solarization×Gravel	1	41.4	41.4	27.8	< 0.001	***
	Residuals	12	17.9	1.5			
$A_{upper} + A_{lower}$	Solarization	1	169.9	169.9	43.9	< 0.001	***
	Solarization×Gravel	1	190.4	190.4	49.2	< 0.001	***
	Residuals	15	58.1	3.9			
d	Trial	2	132.2	66.1	184.3	< 0.001	***
	Solarization	1	72.1	72.1	200.9	< 0.001	***
	Solarization×Gravel	2	20.9	10.4	29.1	< 0.001	***
	Residuals	12	4.3	0.4			

[z]T_{ave}: Average daily mean temperature, A_{upper}: upper amplitude, A_{lower}: lower amplitude, d: damping depth in eq.2.

[y]Trial = Trial 1 and 2 in San Rafael, CA, and in Corvallis, OR; Solarization = solarized or non-solarized treatment; Gravel = 0, 2.5, or 7.5 cm gravel thickness treatment.

plots indicates that amplitude stays large at greater depths compared to that in non-solarized profile. This implies that a larger amount of heat is conducted to deeper horizons in solarized plots than in non-solarized plots.

Addition of gravel layers generally lowered T_{ave} in non-solarized plots (0.33 C per cm-gravel); however, it increased T_{ave} in solarized plots (0.32 C per cm-gravel), and a significant interaction between solarization and gravel depth was indicated (Fig. 3, Table 1 and 2). It also increased amplitude by 1.61 C per cm-gravel in solarized plots but did not affect non-solarized plots (P = 0.334, ANOVA). Damping depth, d, did not significantly differ among gravel treatments in non-solarized plots (P = 0.415, ANOVA), but was reduced by the increasing gravel thickness in solarized plots (0.28 cm per cm-gravel) (P = 0.007).

The gravel layer has a smaller heat capacity, and maybe less heat conductivity than soil. The volumetric specific heat of quartz, clay minerals, organic matter, water, and air are 2.13, 2.39, 2.50, 4.18, and 0.0012 MJ m^{-3} K^{-1}, respectively (de Vries 1963). The gravel layer is mainly

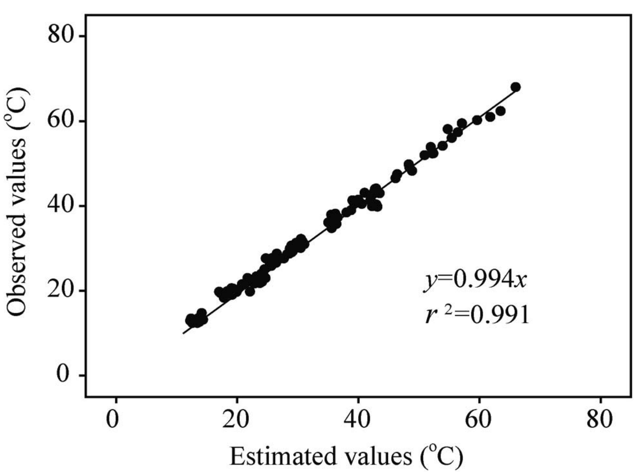

Fig. 2. Comparison of estimated values from the average maximum and minimum daily temperature model (equation 2) with estimated parameters from the reduced model and the observed values in field trials. Regression line and coefficient of relation (r^2) are shown.

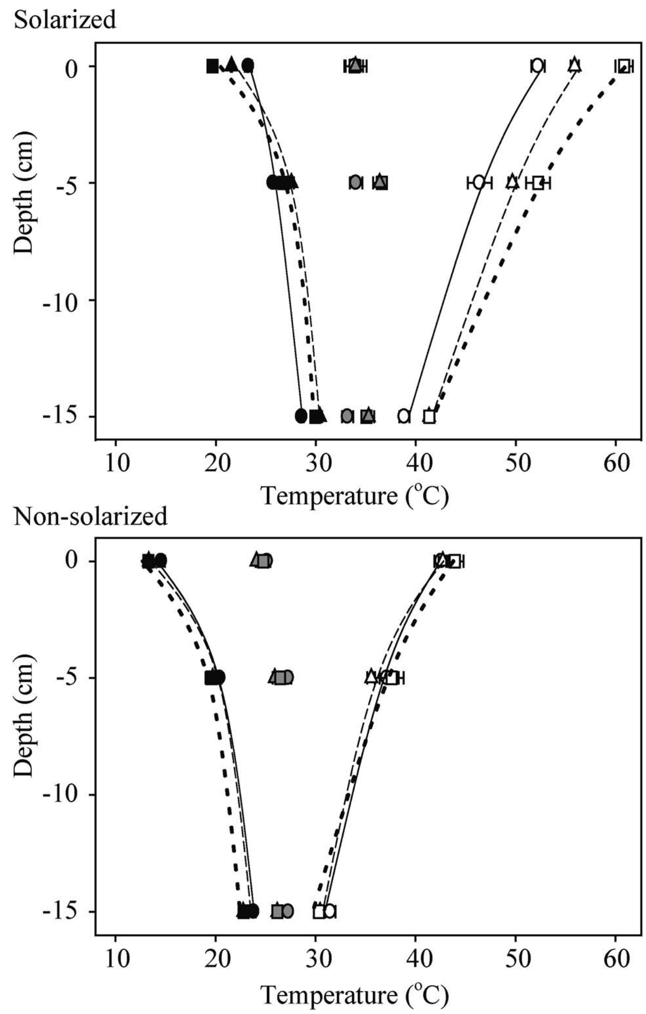

Fig. 3. The effect of different gravel thickness treatments (circle: 0 cm, triangle: 2.5 cm, square: 7.5 cm) on average maximum (open symbols), minimum (filled symbols), and mean average (gray symbols) daily temperature in different depths in solarized and non-solarized plots in trial 2 in CA. Non-linear regression lines are shown for each gravel treatment (solid lines: 0 cm gravel, dashed lines: 2.5 cm gravel, dotted lines: 7.5 cm gravel thickness). Similar trends were observed in other trials.

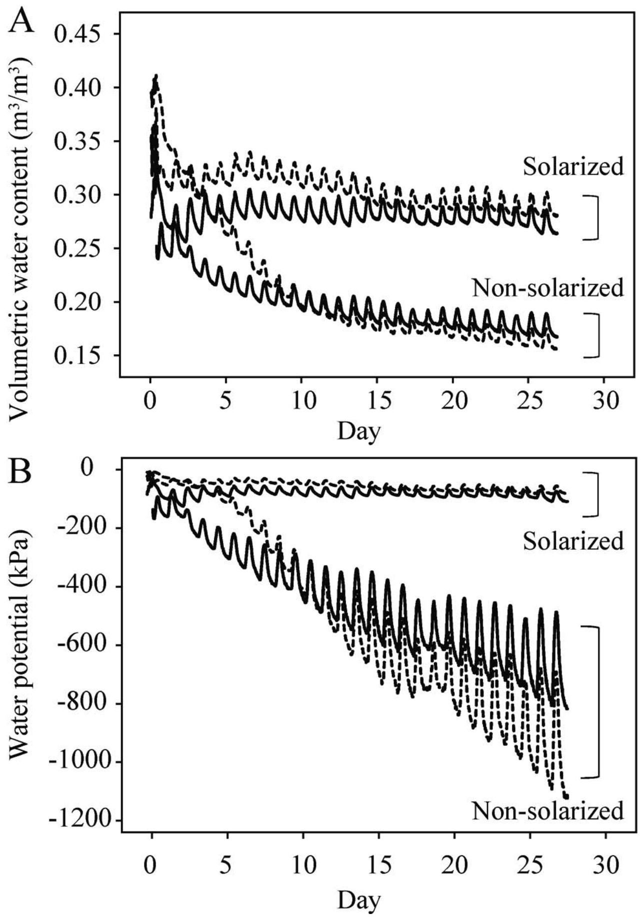

Fig. 4. Volumetric water content measured by soil water content reflectometers (A) and converted water potential (B) at 5 cm depth over four weeks of solarization treatment in trial 2, CA (solid lines: 0 cm gravel, broken lines: 2.5 cm gravel thickness). Similar trends were observed in other trials.

composed of quartz and air that have less specific heat than other components. Therefore, the total heat capacity of the gravel layer would be smaller than the soil layer. A smaller heat capacity indicates that the temperature of the gravel layer would rapidly increase during daytime (Hillel 1998). The same process happens during nighttime where the temperature of the gravel layer quickly decreases at the surface. On the other hand, thermal conductivity is less predictable. The thermal conductivity of quartz, clay minerals, organic matter, water, and air is 8.80, 2.92, 0.25, 0.57, and 0.025 $W \cdot m^{-1} \cdot K^{-1}$, respectively (de Vries 1963). While individual grains of gravel may have high conductivity, the air phase surrounding the gravel and the low contact area between gravel particles is likely to yield a low overall thermal conductivity. It is known that soils with high air content and lower water content have lower thermal conductivity (de Vries 1975, Hillel 1998). Because gravel-sand mulch was reported to have a lower thermal conductivity compared to soil (Li 2003), the gravel layer in our study might also have had a smaller thermal conductivity, especially when it was dry.

In solarized plots, the greater temperature amplitudes in solarized plots with gravel are believed to be due to the smaller heat capacity of the gravel layer, which is consistent with results from a simulation study (Lu et al. 2013). The increased average temperature by gravel treatments is likely due to lower latent heat loss. In solarized plots where there is less air movement compared to non-solarized plots, the gravel layer with less thermal conductivity may have served as thermal insulation, retaining heat during the night (Li 2003). This is evident by a larger amplitude at the surface but significantly decreased value of d with increased gravel thickness, which describes more rapid decrease in temperature amplitude with depth, which would result from a smaller thermal conductivity of the gravel layer relative to soil.

In non-solarized plots, increasing the gravel thickness slightly lowered the average temperature, and the amplitude was similar across gravel thicknesses. This is in contrast to previous studies with gravel mulch (Lu et al. 2013, Li 2003, Nachtergaele et al. 1998). The heat loss in our system may be due to high porosity of the gravel layer resulting from the uniform 1.9 cm gravel in our trials rather than the mixed gravel and sand (Lu et al. 2013, Li 2003) or non-porous limestone fragments (diameters ranging between 2 cm and 8 cm) (Nachtergaele et al. 1998) used in other studies because increased porosity of the mulch decreases the heat storage function (Xie et al. 2010).

Soil volumetric water content (VWC) was consistently greater in solarized plots than in non-solarized plots during the field trials at both the 5 and 15 cm depth (Fig. 4 and 5) as revealed by a significantly smaller estimated parameter λ in solarized vs. non-solarized plots (Table 1 and 3). VWC was significantly lower at the 5 cm depth than the 15 cm depth in non-solarized plots, indicating the presence of an evaporation gradient towards the surface, and quantified by a smaller estimated λ ($P = 0.013$, ANOVA, Table 3) in contrast to solarized plots where λ did not significantly differ between depths ($P = 0.120$, ANOVA, Table 3). Parameter λ also did not significantly differ among gravel treatments ($P = 0.208$, ANOVA).

During the 4-week trials, the soil water potential in solarized plots mostly stayed higher than -100 kPa at both 5 and 15 cm depths (Fig. 4B and 5B). In contrast, the soil water potential in non-solarized plots reached up to -1000 kPa at the 5 cm depth and -400 kPa at 15 cm in non-solarized plots. The water potential in leaf disks quickly decreased within the first 5 days in all treatments and achieved -15.0 to -33.0 MPa (Fig. 6). There was no significant difference in the leaf disk water potential among gravel treatments at the 0 cm depth ($P = 0.498$, ANOVA). At the 5 cm depth, measured only in the gravel layer that was 7.5 cm in thickness, the leaf disk water potential decreased significantly slower than at the 0 cm depth in solarized plots (Fig. 6, $P < 0.001$, ANOVA). Our result showed that leaves at the surface or in the gravel layer became dried in non-solarized plots as well as solarized plots at least during the daytime when the temperature achieved the critical condition for the inoculum. The slower decrease in water potential at the 5 cm depth in solarized plots with the 7.5 cm gravel treatment should be due to the lower temperature than at the surface. The experiment was not designed to investigate the leaf water

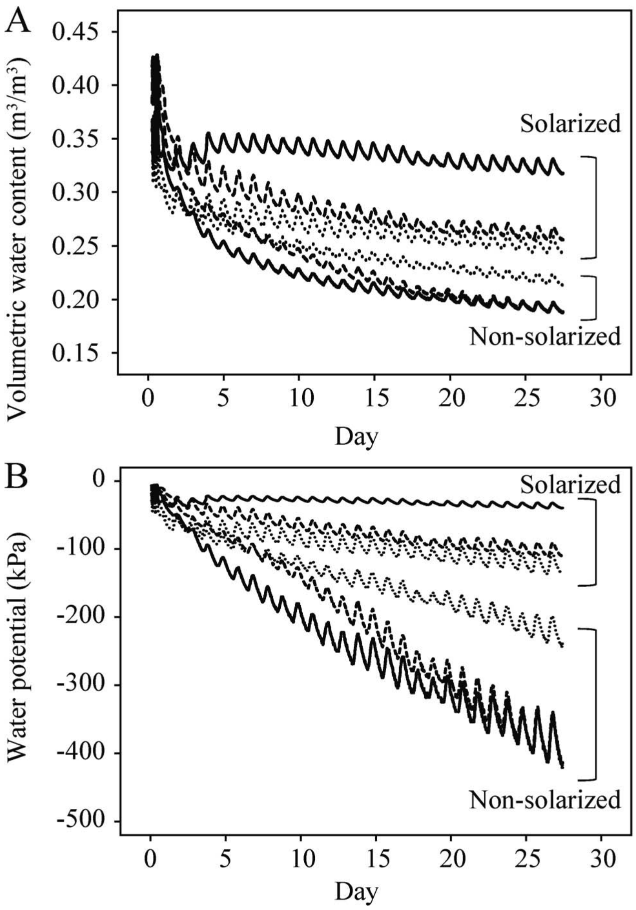

Fig. 5. Volumetric water content measured by soil water content reflectometers (A) and converted water potential (B) at 15 cm depth over four weeks of solarization treatment in trial 2, CA (solid lines: 0 cm gravel, broken lines: 2.5 cm gravel, dotted lines: 7.5 cm gravel thickness). Similar trends were observed in other trials.

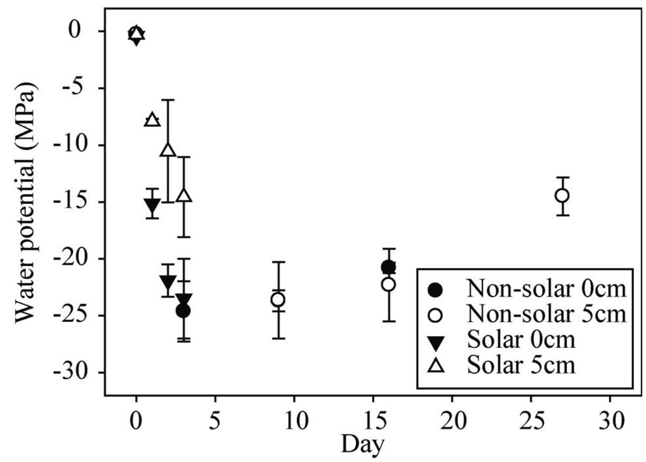

Fig. 6. The water potential of leaf disk inoculum in different depths and gravel treatments during trial 2, CA. The water potential at 5 cm was only measured in the 7.5 cm gravel treatment. Since no significant gravel thickness effect was observed, samples from all three gravel treatments were pooled together. Means ± standard error. Similar trends were observed in other trials.

potential during nighttime because samples were always collected during the daytime.

The *Phytophthora* inoculum at all depths was killed in solarized plots within four weeks in all trials. Only data from trial 2 in CA is shown in Fig.7. In comparison, inoculum was recovered in non-solarized plots over four weeks except at the 0 cm depth. In trial 1 and 2 in CA, slightly but significantly longer survival was observed at the 15 cm depth in the solarized plots with the 0 cm gravel treatment than in plots with the 2.5 cm or 7.5 cm gravel treatment (Fig. 7, Table 4). In our system, however, we did not observe any significant difference in the inoculum recovery between the 2.5 cm and 7.5 cm gravel thicknesses. Also, there was no statistically significant difference observed in inoculum recovery at a 5 cm depth among gravel treatments in solarized plots (Table 4). The comparison of two different *Phytophthora* species in trial 2 in CA showed slightly, but significantly longer survival of *P. pini* than *P. ramorum* inoculum at the 5 and 15 cm depths (Fig. 8 and Table 4).

Solarization effects on the survival of *Phytophthora* inoculum were also consistent with our previous study (Funahashi and Parke 2016). Slightly longer survival at the 15 cm depth in the solarized plots with the 0 cm gravel treatment than in plots with other gravel treatments in trial 1 and 2 in CA reflected the higher daytime soil temperature regime in the solarized plots with gravel than in plots

Table 3. ANOVA table of the reduced model to estimate the proportionality constant (λ) in volumetric water content model (equation 3). AIC and ANOVA comparisons were used to achieve the reduced model for each parameter.

Data set[z]	Effect[y]	Df	Sum Sq	Mean Sq	*F* value	*P* value	
0 cm and 2.5 cm gravel							
	Solarization	1	13353	13353	28.9	< 0.001	***
	Trial	2	5350	2675	5.8	0.011	*
	Depth	1	1228	1228	2.7	0.120	
	Solarization×Depth	1	3526	3526	7.6	0.013	*
	Residuals	18	8318	462			
15 cm depth							
	Solarization	1	6455	6455	12.2	0.004	**
	Trial	2	3953	1976	3.7	0.050	*
	Residuals	14	7381	527			

[z]The effects of trial, solarization, depth, and gravel treatment on constant λ were tested by using a general linear model on samples from 0 and 2.5 cm gravel treatment plots. Similarly, the effects of trial, solarization, and gravel treatment on constant λ were tested on samples at 15 cm depth from all plots.

[y]Trial = Trial 1 and 2 in San Rafael, CA, and in Corvallis, OR; Solarization = solarized or non-solarized treatment; Gravel = 0, 2.5, or 7.5 cm gravel thickness treatment.

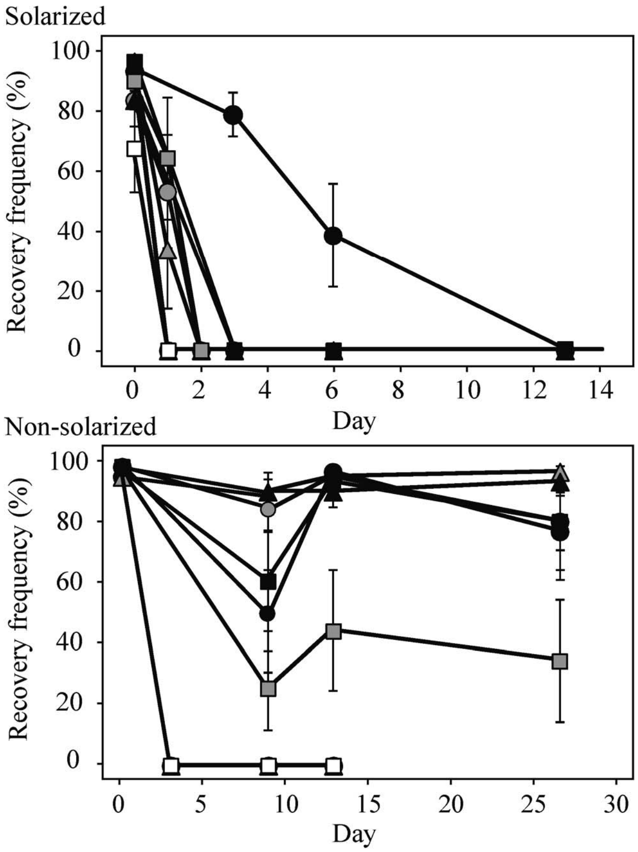

Fig. 7. Recovery frequency of *P. ramorum* inoculum at different depths (open symbols: 0 cm, grayed symbols: 5 cm, closed symbols: 15 cm) and gravel thickness treatments (circle: 0 cm, triangle: 2.5 cm, square: 7.5 cm) in trial 2, CA during solarization (A: solarized, B: non-solarized). Means ± standard error. Similar trends were observed in other trials.

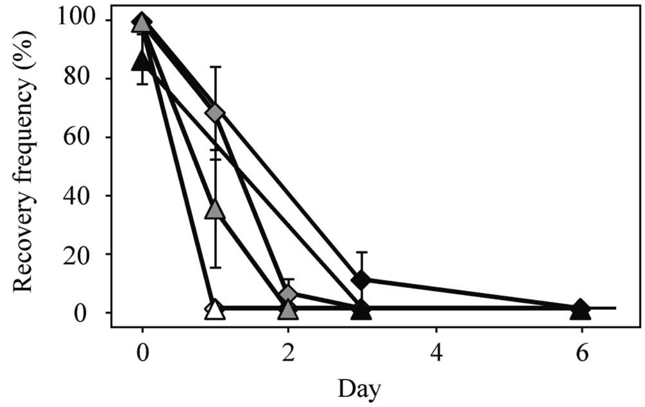

Fig. 8. Recovery frequency of *P. ramorum* (triangle) and *P. pini* (diamond) inoculum at different depths (open symbols: 0 cm, grayed symbols: 5 cm, closed symbols: 15 cm) in solarized treatment in trial 2, CA. The plots were covered with a 2.5-cm-thick gravel layer. Means ± standard error.

without gravel. In our system, however, we did not observe any significant difference in the inoculum recovery between the 2.5 cm and 7.5 cm gravel thicknesses, probably because the sampling resolution (1 day as finest) was not small enough to detect the difference. Since the longer survival of *P. pini* than *P. ramorum* was consistent with previous studies (Werres et al. 2001, Jung and Burgess 2009, Funahashi and Parke 2018), the heat condition where *P. pini* is killed can be assumed to also be lethal for *P. ramorum*. Therefore, *P. pini* can be used as an indicator of lethal conditions of solarization for *P. ramorum*.

Many pathogens have been reported to be more heat resistant at lower water potentials (e.g. Shlevin et al. 2003, Shlevin et al. 2004, Usmani and Ghaffar 1986). *P. ramorum* was also reported to be more heat tolerant in dry heat than wet heat (Schweigkofler et al. 2014). Pathogen inoculum in non-solarized plots, or inoculum at the surface or in a gravel layer in solarized plots where water potential was recorded to be relatively low would have been significantly more resistant to heat. The similar survival of the inoculum at a 5 cm depth among all gravel treatments during solarization (Fig. 7, Table 4) might indicate the complex interaction of increased temperature and reduced water potential by the gravel treatment. The inoculum at a 5 cm depth in plots with 7.5 cm gravel was located within the gravel layer that experienced more intense heat and lower water potential in contrast to inoculum at a 5 cm depth in other gravel treatments that were located within the soil layer. The potentially extended survival of the inoculum by being in a dry condition might have cancelled out the effect of increased heat. Based on our result, the 2.5 cm gravel treatment might be sufficient to enhance the effect of the soil solarization across the soil profile, at the same time minimizing the thickness of the drier layer where the inoculum becomes more tolerant to the heat. It will be still important to investigate the heat susceptibility of inoculum at different water potentials.

In conclusion, our study demonstrated that the addition of a gravel layer to the soil surface results in increased temperature at all depths during solarization. In soil that is kept moist, the efficacy of solarization in killing *Phytophthora* inoculum is enhanced relative to soil that is dry.

Table 4. *P* values of solarization, gravel, and inoculum effects from ANOVA on recovery (%) of *P. ramorum* and *P. pini* from infested leaf disks buried at 0, 5, and 15 cm depths in trial 1 and trial 2 in San Rafael, CA, and in Corvallis, OR.

	CA trial 1			CA trial 2			OR		
Treatment	**0 cm**	**5 cm**	**15 cm**	**0 cm**	**5 cm**	**15 cm**	**0 cm**	**5 cm**	**15 cm**
Solarization[z]	0.386	< 0.001	< 0.001	0.71	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Gravel[z]	0.439	0.820	0.002	0.988	0.566	< 0.001	0.193	0.128	0.525
Inoculum[y]	-	-	-	1	0.004	< 0.001	-	-	-

[z]*P* values are shown associated with Day×Solarization for solarization treatment and Day×Gravel for gravel treatment from ANOVA based on the model of logit(Recovery) = Intercept+Day+Day×Solarization+Day×Gravel+Day×Solarization×Gravel (n = 6).

[y]*P* values are shown associated with Day×Inoculum from ANOVA based on the model of logit(Recovery) = Intercept+Day+Day×Inoculum (n = 6).

However, within a gravel layer, leaf inoculum appeared to have a significantly lower water potential than in soil. This illustrates the complex interaction in gravel between increased heat tolerance of the inoculum when in a dry condition, co-occuring with exposure to increased temperatures. It is unknown how deep in the soil horizon infested leaf debris infiltrates gravel layers as compared to bare soil. In bare soil, *Phytophthora* spp. were detected up to 15 cm (Dart et al. 2007). It is more likely that inoculum reaches a deeper horizon in gravel-treated ground because of the increased permeability of the gravel layer and the high water content sustained in the soil layer underneath. At the same time, it is less likely for the pathogen to infect container plants placed above the gravel layer because there would not be a continuous water pathway for zoospore movement. Further research is necessary to clarify the pathogen distribution in field soil under a gravel layer and determine its ability to infect container plants.

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