Field Evaluation of a Novel, Granular Soil Fumigant for Controlling *Phytophthora ramorum* in Field Nursery Soils

Marissa L. Layman[1], Craig Ramsey[2], Wolfgang Schweigkofler[3] and Steven E. Newman[4,*]

[1]*Independent Research Associate*

[2]*Retired USDA-APHIS Fort Collins, Colorado 80526, USA*

[3]*National Ornamentals Research Site at Dominican University of California, San Rafael, California, 94901, USA*

[4]*Colorado State University, Horticulture and Landscape Architecture, Fort Collins, CO, 80523, USA*

Abstract: *Phytophthora ramorum*, the causal agent of Sudden Oak Death (SOD) and ramorum blight, infects a wide range of hardwood and nursery ornamental species. Chlamydospores of *P. ramorum* can survive for extended periods of time in soils. Two studies were conducted, including: 1) a laboratory study to evaluate two liquid disinfectants for controlling *P. ramorum* chlamydospores, and 2) a field study to evaluate a novel soil fumigation treatment as an alternative to soil steaming or methyl bromide soil fumigation. The liquid disinfectants were ElectroBiocide and Oxidate 2.0. The laboratory study resulted in complete inactivation of the *P. ramorum* chlamydospores after six minutes of contact time for both the liquid disinfectants The field study evaluated a chlorine dioxide granule formulation that was applied at two rates in a nursery soil. Rhododendron leaf discs were inoculated with *P. ramorum*, placed in permeable sachets and buried at two soil depths in a research nursery. Soil treatments also included saturated hydrogels (with and without gels) so that soil moisture effects on chlamydospore survival could be estimated. The sachets were recovered 5, 15 and 30 days after the soil treatment. Efficacy of the soil treatments was evaluated by the number of leaf discs showing *P. ramorum* growth recovered from the sachets. The soil fumigation treatment with highest efficacy occurred when the sachets were buried at the 5 cm soil depth, were treated with hydrogels, at the highest Z-series granule rate (800g/tube), and had a contact time of 30 days. The probability of *P. ramorum* growth for this soil treatment was 0.18, or 18%, i.e. the probability of that fumigation treatment inactivating the pathogen was 82%. Also, as the soil moisture increased, the efficacy of the fumigation treatments also increased.

Keywords: *Phytopthora ramorum*, Soil Fumigation, Chlorine Dioxide Granules, Nurseries.

1. INTRODUCTION

Phytophthora ramorum is a plant pathogen that causes Sudden Oak Death and ramorum blight in forests and woody plants [1-3]. It was first identified in the United States in 2001 in coastal California on native forest trees (esp. tanoak *Notholithocarpus densiflorus* and Coast live oak *Quercus agrifolia*) and also among the infected woody ornamental plants in nurseries. Federal regulations were put in place in order to stop the spread of the pathogen through plant trade [4, 5]. *P. ramorum* is considered an invasive species in the USA based on its spreading pattern and limited genetic variability. The pathogen has the ability to spread aerially, has multi-year persistence in a range of climatic conditions, and reproduces quickly in a variety of environments. Many different plant species are foliar hosts, such as California bay laurel (*Umbellularia californica*) and *Rhododendron spp*. [6].

P. ramorum produces chlamydospores, but not oospores like other *Phytophthora* species. These survival structures can survive in soils for several years. Widmer [7] found that 78% of *P. kernoviae* oospores survived after being buried in sand, at 30°C, for one year. Also, Babadoost and Pavon [8] found that *P. capsici* oospores were still viable after burial in a soil for three years, but were not viable after 4 years of burial. In a nursery study, Grünwald *et al*. [9] found that *P. ramorum* chlamydospores buried at two different depths in the soil, at 4°C, were still viable up to a year after the burial date.

A global initiative to evaluate alternative soil fumigants has been ongoing since the 1992 Montreal Protocol International treaty, which limited the use of methyl bromide. Very few soil fumigants have been registered by the US Environmental Protection Agency (EPA) since the start of this initiative. The most recent EPA registered soil fumigant uses dimethyl disulfide (DMDS) as the active ingredient and is sold as Paladin® (Arkema, Inc., King of Prussia, PA USA).

Another alternative fumigant to methyl bromide is granulated chlorine dioxide (ClO_2) that is manufactured by ICA TriNova (Newnan, GA. USA). Chlorine dioxide is a strong oxidant that can be used as a broad spectrum biocide to inactivate bacteria, fungi, and

*Address correspondence to this author at the Colorado State University, Horticulture and Landscape Architecture, Fort Collins, CO, 80523, USA; Tel: +1 (970) 491-7118; E-mail: Steven.Newman@colostate.edu

E-ISSN: 2409-9813/20

viruses [10, 11, and 12]. Chlorine dioxide gas is generated from granules by decomposition of a precursor chemical (slow release) or by reactions with an acidic precursor (fast release formulations). Also, chlorine dioxide granules can be developed as controlled release formulations, which release ClO_2 gas over several days. This improves the effectiveness of the fumigant against soil-borne pathogens.

The overarching goal of this field nursery study is to evaluate an alternative soil fumigation method that could replace methyl bromide fumigation. The specific goal of this study is to evaluate a granular chlorine dioxide formulation for the ability to inactivate *P. ramorum* inoculated onto the rhododendron leaf discs, which were buried in the nursery soil.

2. MATERIALS AND METHODS

The field study was conducted at the National Ornamentals Research Site at Dominican University of California (NORS-DUC) research nursery, which is in San Rafael, CA, USA. The soil fumigation study ran for 30 days in June and July, 2017. Prior to the study, the nursery plot was covered with a large tarp in order to prevent rain from moistening the soil. However, after the soil fumigation treatments were applied, the plots were irrigated to mimic winter rainfall patterns, which promote *P. ramorum* growth and survival.

The study has a factorial design with all four study factors fully crossing with one another. The study factors include: three rates of Z-series granules (0, 200, and 400 g/tube), two soil depths for inoculated leaf discs in each tube (5 and 10 cm deep from the soil surface), three soil fumigation time periods (5, 15, and 30 days after starting the fumigation treatments), and two soil hydrogel treatments (tubes with and without hydrogels placed on leaf disc sachets). All treatments were randomized and assigned to the 54 soil tubes that were then labelled. There were 18 control tubes that did not receive the Z-series fumigation treatments.

The field nursery plot was 3.6 x 9.1 m and contained gravelly loam soil (xerorthents urban land complex) originally excavated from the Dominican campus. Within the treatment plot, 54 polyvinyl chloride (PVC) tubes (diam. 25 cm x 30 cm length) were buried 15 cm deep in the soil. Soil was packed around the outside of the PVC tubes to ensure a firm seal between the tube and the soil to prevent leaking of ClO_2 fumigant. At a depth of 28 cm, each tube contained 13.8 L of soil.

Chlorine dioxide granules (ICA TriNova, Newnan, GA, USA) were used for this study. The fumigant granule formulation (Z-series) consisted of two precursor chemistries impregnated onto two different zeolite carriers. The fast release Z-series Part A is the ClO_2 precursor (sodium chlorite, or $NaClO_2$ on a zeolite carrier) and Part B is the acid activator (sodium bisulfate on a zeolite carrier). When the two precursor granules are mixed in soil, the chlorine dioxide gas is controlled-released over approximately a 60 to 80-hour time period. The ClO_2 release rate is dependent on the precursor chemistry, granule weight, and ambient soil moisture and temperature. The precursors are not activated by ambient humidity or high soil moisture, but by the volatilization rate of the acid precursor, which is temperature dependent. Sodium chlorite is also partially activated by soil moisture.

Z-series granules were applied at three rates per granule type (Part A + Part B) at a total weight of 0, 400 or 800 g/tube. Part A and Part B granules were added at equal weights per tube (i.e., for the 400 g/tube pre-weigh 200 g Part A + 200 g Part B granules). The two vials were finally combined together as they were added to the soil while blending in a cement mixer (19 L capacity) for 3 min/soil tube. The ratio of Z-series granules weight to soil volume was either 400 g/13.8 L (0.029 g/ml of soil), or 800 g/13.8 L (0.058 g/ml of soil).

The soil was returned to its respective PCV tube in two layers so that the inoculated leaf sachets could be placed at their treatment depth. The first soil layer was18 cm from the bottom of the tube, and the second soil layer was 23 cm from the bottom of the tube. Inoculated leaf sachets were added to the first and second soil layers, and a 5 cm soil moisture sensor (5EC sensor) (METER Environment, Pullman, WA, USA) was added to an assigned soil layer (5 or 10 cm deep from the soil surface).

P. ramorum isolate Pr-1418886, grown for 3 weeks on V8-juice agar (V8A; 100 mL filtered V8 juice, 0.1 g $CaCO_3$ and 900 mL distilled water; [13]), was used to inoculate leaves from *Rhododendron x* 'Cunningham White' plants maintained at a quarantine site. Infected leaves were placed in a plastic box with damp paper towels and incubated at 20ºC for three weeks. Leaf discs were cut from symptomatic leaves using a 5 mm diameter punch.

Ten leaf discs were placed into 8 x 10 cm permeable nylon sachets. The nylon sachet fabric allowed ClO_2 gas to penetrate and absorb onto the leaf

discs, but also woven tight enough to prevent soil from contaminating the leaf discs. Two sachets were placed at each of the two soil depths (5 or 10 cm deep from the soil surface), for a total of 20 inoculated leaf discs placed at each soil depth. The presence of *P. ramorum* was confirmed by plating 10 leaf discs on selective PARPH-V8 media [14, 15].

Hydrogels (Terra-sorb, Lebanon Seaboard Corp., Lebanon, PA, USA) were placed around the leaf sachets in order to keep the soil moist during the time period between filling the soil tubes and when the irrigation was turned on with the intent of increasing the overall survival rate of *P. ramorum*. Hydrogels were mixed at a rate of 31.1g/500 ml water to make up a total volume of 19 L of swollen hydrogels. Tubes assigned for hydrogel treatments received approximately 780 ml of the hydrated gels, added on top of the two leaf sachets for both soil layers. Control tubes assigned to hydrogel treatments were also given the gels at both soil levels, but without the Z-series granules added to the soil.

All tubes were then covered with a 30 cm clear, acrylic plastic cap (TAP Plastics, Sacramento, CA, USA) sealed with white silicone caulk to prevent any ClO_2 gas escaping from the soil surface. The tube caps were sealed onto each tube for five days after the soil treatments were applied hence mimicking the effects of tarping the soil after a standard soil fumigation treatment. During these first five days, the soil tubes were not irrigated. Once the caps were removed from the tubes, overhead irrigation was turned on.

Overhead irrigation was applied to keep the soil moisture between 10 to 30% (v/v) over the 30-day study period. Soil moisture levels were targeted for average moisture levels in the early spring, optimal for the survival of chlamydospores. Soil moisture data were collected using the 5 cm probes connected to a METER (EM50) data logger (METER Environment, Pullman, WA, USA), which collected data every two hours for the full 30-day study period. The two-hour soil moisture data were averaged for the 5 and 30 day sachet collection dates.

Two sets of control studies were used to measure the viability of *P. ramorum* over time. The field controls were leaf sachets buried in the soil tubes, at both soil depths, with and without the hydrogel treatments, and irrigated the same as the other treatments. The second set of controls were leaf discs left in storage inside the laboratory.

The inoculated leaf sachets were retrieved from the tubes at the three fumigation exposure dates (5, 15, and 30 days after soil treatment). Leaf discs were cultured on PARP-H V8 at 20ºC and evaluated for *P. ramorum* colonies after 14 days.

An additional set of controls tested the ability of Z-series granules to inactivate *P. ramorum*, under lab conditions, without soil. Three Petri plates containing 10 leaf discs were used to run this test. The Z-series granules (0.26g Part A + 0.26g Part B/plate) were added to each plate. The plates were then covered and sealed with parafilm. Plates were exposed to ClO_2 gas for 65 hours before removing the leaf discs for culture and assaying for viable *P. ramorum*.

A second laboratory study evaluated the ability of two liquid disinfectants to inactivate *P. ramorum*, which were inoculated onto cellulose nitrate coupons, that were replicated 12 times per treatment. The two liquid disinfectants were ElectroBiocide (Strategic Resource Optimization, Bailey CO, USA) and Oxidate 2.0 (BioSafe Systems, Hartford, CT, USA). Electrobiocide is a chlorine dioxide formulation and Oxidate 2.0 is a hydrogen peroxide formulation. Electrobiocide and Oxidate 2.0 were applied at 200 and 10,000 ppm, respectively, using a hand bottle sprayer. The three disinfectant contact times were 2, 4, and 6 min per coupon. Oxidate 2.0 was neutralized with sodium bicarbonate and mixed at 100 g/L H_2O. ElectroBiocide was neutralized with sodium thiosulfate and mixed at 25 g/L H_2O. The neutralizer was applied to each coupon at the end of the contact time. All coupons were assayed for *P. ramorum* growth as previously described.

A Design of Experiment program (DOE) (SAS-JMP, SAS Institute, Cary, NC, USA) was used to create the soil tube treatment list. The DOE estimated 19 replications per treatment based on “hidden replication” that occurs by limiting the data analyses to only two-way interactions. This eliminated the need for testing four-way interactions distributed among the four study factors. Viable leaf disc counts, for every sachet, were divided by the ten discs per sachet in order to generate a binomial dataset for *P. ramorum* survival rates. The data were analyzed with a Logistic Regression program (SAS-JMP, SAS Institute Inc., Clary, NC, USA). Logistic regression is the preferred test for a binary survival variable, which has probability values between 0 and 1. Test results were considered significant at α = 0.05.

3. RESULTS

Analysis of the *P. ramorum* viability data shows that the Z-series rates, leaf disc burial depth, and hydrogel status affected the number of *P. ramorum* survival (Table **1**). The number of fumigant exposure days was the only factor that did not affect *P. ramorum* viability. There were no interactions between the four study factors (data not shown). Comparison of the probability of *P. ramorum* survival shows that the most important study factor was the sachet burial depth (Tables **2** and **3**). All the leaf discs that were buried at 10 cm deep from the soil surface had 100% *P. ramorum* survival across all study factors (Table **3**).

The soil fumigation treatment with the highest leaf disc inactivation of *P. ramorum* chlamydospores occurred when leaf discs were buried at 5 cm soil depth, hydrogels were added over leaf disc sachets, Z-series granules were applied at 400 g per tube for Part A and Part B granules, and sachets were exposed to fumigant treatments for 30 days (Table **2**). The expected probability of an inoculated leaf disc to show no *P. ramorum* growth under these conditions was 0.811, or 81%. The probability of an inoculated leaf disc showing no *P. ramorum* growth dropped to 27% when leaf discs were buried at 5 cm and Z-series granule rate was 200 g/tube (Table **2**).

Table 1: Effects of Likelihood Test and Significant Values for Each of the Tested Study Factors for *Phytophthora ramorum* Remaining Active and Inactive

Source	DF	Prob>ChiSq
Leaf Burial Depth (cm)	1	0.0001
Days Post Fumigation (days)	2	0.2124
Hydrogels (Yes or No)	1	0.0477
Granule Rate (g)	2	0.0006

Table 2: Probability Binomial Model of *Phytophthora ramorum* Remaining Active and Inactive at the End of the Three Exposure Times and Two Tested Granular Concentrations with the Presence or Absence of Hydrogels, with Leaf Disc Sachets Buried at 5 cm Below the Soil Surface

Days Post Fumigation	Hydrogels	Granules of Fumigant (g)	Probability *P. ramorum* Growth	Probability *P. ramorum* Inactivation	Percent leaf discs with P. ramorum growth (%)
5	No	0	0.999	7.73E-10	100
5	No	200	0.982	0.019	100
5	No	400	0.818	0.182	.
5	Yes	0	0.999	7.33E-08	100
5	Yes	200	0.849	0.151	100
5	Yes	400	0.321	0.679	0
15	No	0	1	2.27E-10	100
15	No	200	0.994	0.006	100
15	No	400	0.939	0.061	75
15	Yes	0	0.999	2.16E-09	100
15	Yes	200	0.95	0.049	100
15	Yes	400	0.617	0.383	75
30	No	0	0.999	1.57E-09	100
30	No	200	0.963	0.037	100
30	No	400	0.689	0.311	100
30	Yes	0	0.999	1.49E-08	100
30	Yes	200	0.734	0.266	50
30	Yes	400	0.189	0.811	25

Table 3: Probability Binomial Model of *Phytophthora ramorum* Remaining Active and Inactive at the End of the Three Exposure Times and Two Tested Granular Concentrations with the Presence or Absence of Hydrogels, with Leaf Disc Sachets Buried at 10 cm Below the Soil Surface

Days Post Fumigation	Hydrogels	Granules of Fumigant (g)	Probability *P. ramorum* Growth	Probability *P. ramorum* Inactivation	Percent leaf discs with P. ramorum growth (%)
5	No	0	1	0	100
5	No	200	1	1.01E-09	100
5	No	400	0.999	1.2E-09	100
5	Yes	0	1	0	100
5	Yes	200	0.999	9.59E-10	100
5	Yes	400	0.999	1.13E-08	100
15	No	0	1	1.11E-16	100
15	No	200	1	2.97E-11	100
15	No	400	1	3.52E-10	100
15	Yes	0	1	1.11E-16	100
15	Yes	200	1	2.82E-10	100
15	Yes	400	0.999	3.34E-09	100
30	No	0	1	0	100
30	No	200	1	2.05E-10	100
30	No	400	0.999	2.42E-09	100
30	Yes	0	1	0	100
30	Yes	200	0.999	1.94E-09	100
30	Yes	400	0.999	2.3E-08	100

The study design included 18 soil tubes that served as control treatments across all the study factors (18 untreated soil tubes with leaf disc sachets, buried at both 5 and 10 cm, with and without hydrogels, and collected at 5, 15, and 30 days after soil treatment). All 18 control soil tubes had 99.9 to 100% viable leaf discs (Table **2** and **3**). Control discs inoculated with *P. ramorum* that were stored dry in the lab at 5ºC for 5 days were *P. ramorum* positive. In contrast, untreated leaf discs stored at 20ºC for 5 days were overgrown with a dark fungus, which was likely an endophytic or opportunistic species present on the rhododendron leaves.

Soil moisture averaged 9 and 12% (v/v) within the tubes, with and without hydrogels, during the first five days of the study with no overhead irrigation (Figure **1**). Once irrigation began, the soil moisture averaged 20% (v/v) for all soil treatments for the remainder of the 30-day study. Soil moisture ranged from 18 to 24% at the two sachet burial depths and three Z-series rates once overhead irrigation began (Figure **2**). As soil moisture increased from 10 to 30%, the probability of viable leaf discs decreased from 1.0 to 0.7 for the leaf discs recovered at 5 and 30 days (p-value = 0.0189) (Figure **3**).

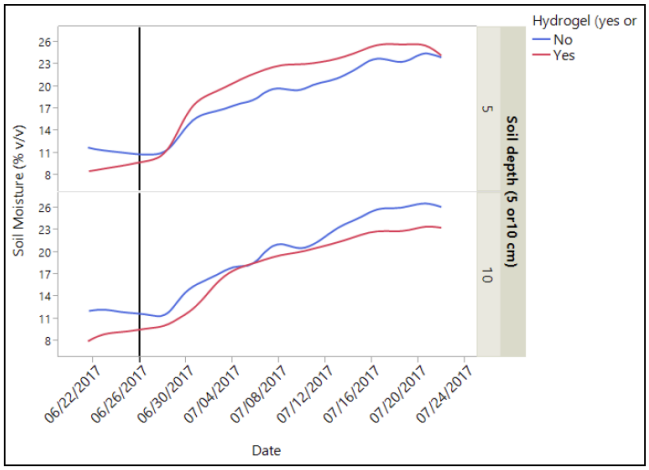

Figure 1: Average soil moisture in soil tubes over 30 days (x-axis) for sachet burial depth (right y-axis) and hydrogel status (legend). Vertical reference line shows five days after fumigant treatments when overhead irrigation turned on for rest of 30-day study.

The Petri plate study tested the effects of ClO_2 gas on the viable leaf discs counts after an exposure time

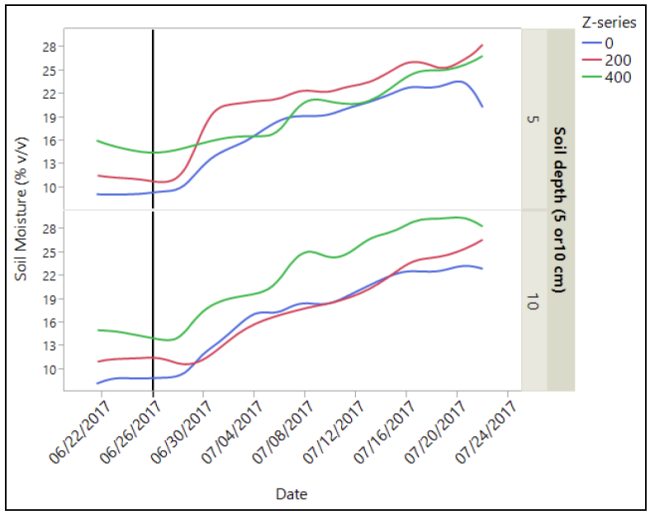

Figure 2: Average soil moisture in soil tubes over 30 days (x-axis) for sachet burial depth (right y-axis) and Z-series granule rates (legend). Vertical reference line shows five days after fumigant treatments when overhead irrigation turned on for rest of 30-day study.

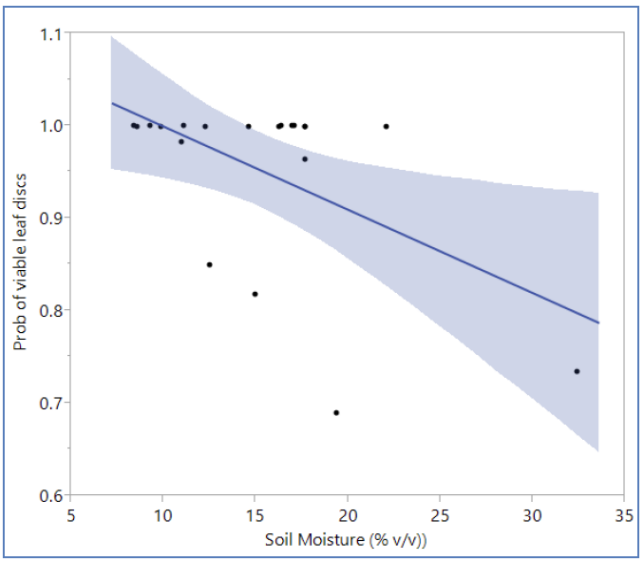

Figure 3: Probability of viable *Phytophthora ramorum*, by soil moisture, for samples collected at 5 and 30 days after soil treatment. The probability data was regressed over average soil moisture for soil treatments for hydrogels, sachet depth, and Z-series rates (p-value = 0.0189).

of 65 hours. The ClO_2 release rates resulted in 100% control for all 30 leaf discs (10 discs per 3 plates) inoculated with *P. ramorum*.

The liquid disinfectant study tested the effects of two oxidants on *P. ramorum* chlamydospore survival rates. *P. ramorum* was completely inactivated after six minutes of contact time with the liquid disinfectants ElectroBiocide and Oxidate 2.0 (Table **4**).

Table 4: Average *Phytophthora ramorum* Counts (CFU/Sample) for Two Disinfectants and Three Disinfectant Contact Times

Disinfectant type	Disinfectant contact time (min)	*Phytophthora ramorum* growth (CFU/sample))
Control	0	75
ElectroBiocide	2	10
ElectroBiocide	4	2
ElectroBiocide	6	0
Oxidate 2.0	2	4
Oxidate 2.0	4	2
Oxidate 2.0	6	0

4. DISCUSSION AND CONCLUSION

Federal regulations require the treatment of nursery soil infested with *P. ramorum* [16]. Heat treatments such as steaming [17] and solarization [18] are highly effective in controlling *P. ramorum* in nursery beds, and biological control using *Trichoderma asperellum* also reduces *P. ramorum* levels [19]. Alternative soil fumigation methods for controlling *P. ramorum* at nursery sites were recently evaluated using registered EPA fumigants [20, 21]. Linderman and Davis [20] tested the effects of steam and metam sodium on *P. ramorum* chlamydospores mixed into two soil types. They found that metam sodium, applied at three rates, resulted in complete inactivation of the chlamydospores. Yakabe and MacDonald [21] also tested the ability of nine fumigants and two soil drenches on *P. ramorum* and reported complete inactivation of chlamydospores in 21 out of 25 soil fumigant treatments. The EPA registered fumigants tested in their study only included one new fumigant (dimethyl disulfide, or DMDS), which is considered more environmentally friendly than the older fumigants. They found that DMDS applied at 224 kg/ha on the top of the soil and tarped for 14 days resulted in 33% reduction in *P. ramorum*, which demonstrates that it was not as effective as the older fumigants.

Despite that three of the four study factors in this experiment reduced *P. ramorum*, the efficacy results were not as high as we predicted due to the large number of leaf discs that had viable *P. ramorum* counts among the treatments. The reduction in *P. ramorum* control from 81% to 27% when Z-series granules rates were reduced from 400 to 200 g/tube indicates that the rate of granules evenly added to the soil in each tube should be greater than 400 g/tube.

The Z-series application rates used in this study were too high to be practical for large scale use and would be cost prohibitive under most conditions. The highest Z-series rate was 0.058 g/ml of soil, which translates into 58 kg/m^3 of soil. If the Z-series granules could be optimized as a soil fumigant with higher microbial efficacy results, then this application rate would be more economically justified for smaller nursery sites.

Soil fumigant treatments with the leaf discs buried at 10 cm had no effect on *P. ramorum* survival rates. One possible explanation for this difference in *P. ramorum* survival by soil depth is that the Z-series granules (0.95 to 1.1 g/cm^3) were less dense than the loamy clay soil (approximately 1.40 to 1.50 g/cm^3). As the granules are added to the soil in the cement mixer, the granules probably moved towards the opening of the mixer due to their lower density, and the denser soil settled towards the bottom of the mixer. As the mixed soil was poured into a five- gallon bucket, the soil at the bottom of the bucket should have a higher percentage of the granules because they moved to the top of the mixer. As the mixed soil was poured from the bucket into each soil tube, the granule stratification mentioned above would be reversed, i.e. the mixed soil with less granules is in the bottom of the tube while the mixed soil with the most granules is near the top of the tube. Thus, more ClO_2 gas would be generated near the top of the tube which would explain why the sachets at the 5 cm soil depth had lower *P. ramorum* survival rates. Stratification of the granules due to their density differences with the soil could be a possible reason for the higher efficacy against *P. ramorum* at 5 cm soil depth. This stratification problem could be corrected by adding granules in two or three layers in future studies. In commercial nursery conditions, the granules would be evenly distributed into nursery soils using a rototiller.

All *P. ramorum* inoculated leaf discs that were not treated with Z-series granules had 100% survival. This was an unexpected finding that shows that harsh environmental conditions, length of sample collection time, or dry/hot soil conditions had little or no impact on *P. ramorum* survival. These findings are in agreement with Widmer [7], Babadoost and Pavon [8], and Grunwal et. al. [9] that *P. ramorum*, when tested at different life stages, could survive in soils for one to three years.

Increased soil moisture levels increased the efficacy of the Z-granules on *P. ramorum* deactivation across all treatments. Soil moisture readily absorbs ClO_2 as a gas and temporarily stabilizes it allowing longer ClO_2 exposure for the plant pathogen. Considering the generation and release mechanisms for ClO_2 from the Z-series granules, the optimum soil moisture for applying the granules appears to be between 5 and 12% (v/v). However, within two to five days of applying the ClO_2 treatment, soil moisture should be increased up to 20 or 30% to improve fumigant efficacy. In order to properly distribute the granules in the soil, the soil moisture should be low enough for semi-moist soil to readily break up into small particles for better mixing results. As soil moisture decreases, air volume in the soil matrix increases, which allows better diffusion of the ClO_2 gas and increased exposure to plant pathogens. However, after the ClO_2 release rates decline then the soil moisture should be increased up to as much as 30% in order to increase the effectiveness of the fumigant.

The two oxidant-based, liquid disinfectants were effective against *P. ramorum* in the laboratory study. The liquid formulation of chlorine dioxide applied at 200 ppm for six minutes resulted in complete inactivation of *P. ramorum*. These results, along with the Z-series laboratory results, provide evidence that chlorine dioxide can inactivate the *P. ramorum* chlamydospores when applied as either a liquid or gas formulation.

The Z-series granules show promise as an alternative to methyl bromide when used as a soil fumigant. A higher rate may have proven more effective for killing *P. ramorum* on infected leaves. Future research is needed in order to gain a better understanding of using granular chlorine dioxide as a soil fumigant to deactivate *P. ramorum* and other plant pathogens in field nurseries.

ACKNOWLEDGEMENTS

We would like to thank Joel Tenney of ICA TriNova for his advice and expertise in the formulation of the Z-Series disinfectant; Dr. Supriya Sharma (NORS-DUC) for assistance with *P. ramorum* growth and Vernon Huffman (NORS-DUC) for help with setting up the soil research plot. The National Ornamentals Research Site at the Dominican University of California is funded by grants from the 2008, 2014 and 2018 Farm Bills, and administrated through the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) Center for Plant Health Science and Technology (CPHST).

REFERENCES

[1] Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike, ST. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis 2002a; 86(3): 205-215. https://doi.org/10.1094/PDIS.2002.86.3.205

[2] Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST. *Phytophthora ramorum* and Sudden Oak Death in California: Host Relationships. In: Standiford R, McCreary D. 5th Symposium on California Oak Woodlands. USDA Forest Service. Gen Tech 2002b; PSW-GTR-184: 733-740.

[3] Garbelotto M, Davison JM, Ivors K, Maloney PE, Hüberli D, Koike ST, Rizzo DM. Non-oak native plants are main hosts for sudden oak death pathogen in California. California Agriculture 2003; 57(1): 18-23. https://doi.org/10.3733/ca.v057n01p18

[4] California Oak Mortality Task Force (COMTF). September Report. http://www.suddenoakdeath.org/library/ California Oak Mortality Task Force 2017a; p. 5.

[5] Kliejunas JT. Sudden oak death and *Phytophthora ramorum*: a summary of literature. Gen Tech Report- Pacific Southwest Research Station. USDA Forest Service 2010; No. PSW-GTR-234. https://doi.org/10.2737/PSW-GTR-234

[6] Grünwald NJ, Garbelotto M, Huengens K, Prospero S. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends Microbiol 2012; 20(3): 131-138. https://doi.org/10.1016/j.tim.2011.12.006

[7] Widmer T. Survival of *Phytophthora kernoviae* oospores, sporangia, and mycelium. New Zealand J Forestry Sci 2011.

[8] Babadoost M, Pavon C. Survival of Oospores of *Phytophthora capsici* in Soil. Am Pythopatho Soc 2013; 97(11): 1478-1483. https://doi.org/10.1094/PDIS-12-12-1123-RE

[9] Grünwald NJ, Parke J, Peterson E. Development and application of an epidemiological framework for management of *Phytophthora ramorum* on rhododendron in nursery settings. *In* Dominican University, National Ornamental Research Site (NORS-DUC) FY14-15 Annual Report 2015; pp. 54-59.

[10] Bernarde MA, Israel BM, Olivieri VP, Granstrom ML. Efficiency of chlorine dioxide as a bactericide. Appl Microbiol 1965; 13(5): 776-780. https://doi.org/10.1128/AEM.13.5.776-780.1965

[11] Chatuev BM, Peterson JW. Analysis of the sporicidal activity of chlorine dioxide disinfectant against *Bacillus anthracis* (Sterne strain). J Hosp Infect 2010; 74(2): 178-183. https://doi.org/10.1016/j.jhin.2009.09.017

[12] Bang J, Hong A, Kim H, Beuchat LR, Rhee MS, Kim Y, Ryu JH. Inactivation of *Escherichia coli* O157: H7 in biofilm on food-contact surfaces by sequential treatments of aqueous chlorine dioxide and drying. Int J Food Microbiol 2014; 191: 129-134. https://doi.org/10.1016/j.ijfoodmicro.2014.09.014

[13] Erwin CD, Ribeiro OK. Phytophthora Diseases Worldwide. St. Paul, MN: American Phytopathological Society Press 1996; p. 562.

[14] Jeffers SN, Martin SB. Comparison of Two Media Selective for *Phytophthora* and *Pythium* Species. Plant Disease 1986; 70: 1038-1043. https://doi.org/10.1094/PD-70-1038

[15] Ferguson AJ, Jeffers SN. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Disease 1999; 83: 1129-1136. https://doi.org/10.1094/PDIS.1999.83.12.1129

[16] USDA. *Phytophthora ramorum* Confirmed nursery protocol 2014. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/phytophthora-ramorum/ct_protocols

[17] Schweigkofler W, Kosta K, Huffman V, Sharma S, Suslow K, Ghosh S. Steaming inactivates *Phytophthora ramorum*, causal agent of Sudden Oak Death and ramorum blight, from infested nursery soils in California. Plant Health Progress 2014; 15: 43-47. https://doi.org/10.1094/PHP-RS-13-0111

[18] Funahashi F, Parke JL. Effects of soil solarization and *Trichoderma asperellum* on soilborne inoculum of *Phytophthora ramorum* and *Phytophthora pini* in container nurseries. Plant Disease 2016; 100: 438-443. https://doi.org/10.1094/PDIS-04-15-0453-RE

[19] Widmer TL, Johnson-Brousseau S, Kosta K, Ghosh S, Schweigkofler W, Sharma S, Suslow K. Remediation of *Phytophthora ramorum*-infested soil with *Trichoderma asperellum* isolate 04-22 under ornamental nursery conditions. Biological Control 2018; 118: 67-73. https://doi.org/10.1016/j.biocontrol.2017.12.007

[20] Linderman R, Davis E. Eradication of *Phytophthora ramorum* and other pathogens from potting medium or soil by treatment with aerated steam or fumigation with metam sodium. Hort Technology 2008; 18(1): 106-110. https://doi.org/10.21273/HORTTECH.18.1.106

[21] Yakabe L, MacDonald J. Soil treatments for the potential elimination of *Phytophthora ramorum* in ornamental nursery beds. Plant Disease 2010; 94(3): 320-324. https://doi.org/10.1094/PDIS-94-3-0320

Received on 10-02-2020 Accepted on 15-03-2020 Published on 13-11-2020

DOI: https://doi.org/10.15377/2409-9813.2020.07.2