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# Eradication of *Phytophthora ramorum* and Other Pathogens from Potting Medium or Soil by Treatment with Aerated Steam or Fumigation with Metam Sodium

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SUMMARY. *Phytophthora ramorum* survived in potting media infested with sporangia or chlamydospores, allowing the pathogen to remain undetected while disseminated geographically. Chlamydospores or oospores of *P. ramorum*, *Pythium irregulare*, *Thielaviopsis basicola*, and *Cylindrocladium scoparium* produced in vermiculite culture were used to infest potting media. Infested media in plastic plug flats were treated with aerated steam mixtures from 45 to 70 °C for 30 min. In a second experiment, infested media were fumigated in polyethylene bags with a concentration series of metam sodium ranging from 0.25 to 1.0 mL·L$^{-1}$. Survival of the pathogens was determined by selective baiting or direct plating the infested media on PARP selective medium. Assays indicated that all pathogens in the infested potting media were killed by aerated steam heat treatments of 50 °C or higher. Metam sodium concentrations of 1.0 mL·L$^{-1}$ of medium or greater also eradicated all pathogens from the potting medium and soil. These results show that aerated steam treatment or fumigation with metam sodium can effectively sanitize soil-less potting media infested with *P. ramorum* or other soilborne pathogens, as well as *P. ramorum*-infested soil beneath infected plant containers. In addition, steam treatments to 70 °C did not melt plastic plug trays.

The discovery of the ramorum blight pathogen, *Phytophthora ramorum,* infecting many ornamental plants in nurseries and landscapes in several European countries, the United States, and Canada, underscores the threat that this pathogen poses to the nurseries (Osterbauer et al., 2004). An increasing number of plant species have been found to be hosts for *P. ramorum,* especially those in the Ericaceae, Fagaceae, and Caprifoliaceae (Davidson et al., 2005; Goheen et al., 2002; Linderman et al., 2006; Parke et al., 2004; Rizzo et al., 2002, 2005; Tooley et al., 2004; Werres et al., 2001). The host list is even greater considering all the plants with which *P. ramorum* has been associated or shown to be susceptible by artificial inoculation (Linderman et al., 2006). Furthermore, P. *ramorum* has been shown (Linderman and Davis, 2006; Shishkoff and Tooley, 2004) to survive in potting media infested by sporangia or chlamydospores (simulating inoculum that could be produced on infected aboveground tissue), thus underscoring the threat that the pathogen could infest media and remain undetected while being disseminated geographically. Growth media potentially infested with this or other soilborne pathogens, along with contaminated used containers, could be a source of inoculum to initiate infections on a wide range of susceptible plants (Jones and Benson, 2001). In addition, Jeffers (2005) demonstrated the survival of *P. ramorum* in medium around containers of infected plants in a nursery, thus requiring some treatment of the soil to eradicate this regulated, quarantined pathogen.

Growers currently attempt to decontaminate used containers by pressure washing or chemical sanitization. Many simply apply fungicides during the production cycle to prevent infections or to respond to occurrence of diseases. However, pathogens could be eradicated from soil or soil-less media by heat from steam, composting, or solarization, or by chemical fumigation (Baker, 1957; Jones and Benson , 2001). The use of aerated steam to pasteurize soil or potting mixes to eradicate soilborne pathogens, weed seeds, and insects was pioneered by K.F. Baker (1957). The principles of treating soil or potting media with heat at temperatures that would be lethal to pathogens without killing all microorganisms, some of which might be beneficial as antagonists remaining after treatment, were useful to nurseries where chemical eradication of soilborne pathogens was not feasible. Baker (1957) emphasized that air-steam pasteurization of soil or potting media in a range of 60 to 71 °C had the additional advantages (compared with using steam at 100 °C) of 1) reduced chance of destroying microorganisms antagonistic to plant pathogens and therefore leaving a biological buffer to block invasion of the medium by pathogens or the development of "weed" fungi that are activated by high temperatures; 2) reduced risk of developing soil toxins resulting from excessive heating; 3) reduced use of steam and therefore energy; 4) taking less time to

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**Units**

| To convert U.S. to SI, multiply by | U.S. unit | SI unit | To convert SI to U.S., multiply by |
|---|---|---|---|
| 10 | % | mL·L$^{-1}$ | 0.1 |
| 29.5735 | fl oz | mL | 0.0338 |
| 3.7854 | gal | L | 0.2642 |
| 2.54 | inch(es) | cm | 0.3937 |
| 25.4 | inch(es) | mm | 0.0394 |
| 16.3871 | inch$^3$ | cm$^3$ | 0.0610 |
| 1 | ppm | mg·L$^{-1}$ | 1 |
| (°F – 32) ÷ 1.8 | °F | °C | (1.8 × °C) + 32 |

treat and less time before the medium could be used; 5) less working hazard; 6) less moisture condensation in the medium; and 7) no damage to plastic containers.

The use of aerated steam mixtures at pasteurization temperatures has not been considered by most nurseries as a means of sanitizing contaminated containers. However, many nurseries have used hot-water dip tanks or even chemical baths in attempts to eliminate pathogens, often, if not usually, unsuccessfully. On the other hand, aerated steam is used at some nurseries to treat potting media, but usually not for decontaminating flats or other containers that might carry pathogens from previous crops. In recent years, we have noted that some growers have successfully used live steam injected into fabricated chambers, thereby diluting with air to create lower temperature air-steam mixtures that sanitize pots or flats.

It is well known that chemical fumigants can be used to treat soil as a pre-plant means of eliminating soilborne pathogens, insects, and weeds. Highly volatile fumigants such as methyl bromide or chloropicrin, however, must be injected into the soil and tarped to reduce gas escape. They are too toxic and volatile to be used easily to eradicate pathogens from soil-less media or contaminated containers unless they are confined in rooms such as would be used to fumigate fruit for insect control. Metam sodium, on the other hand, is more easily applied in a liquid form as a drench, thereafter releasing methyl isothiocyanate (MIT) as the toxic fumigant. Although it is known that MIT can kill most soilborne fungal pathogens, this has not been demonstrated for *P. ramorum.* However, it is conceivable that metam sodium could be applied efficiently and relatively inexpensively to soil-less media in a containment structure or groundbed to eliminate soilborne pathogens. Furthermore, it could be drench-applied to sanitize areas of nurseries from which containers with infected plants had been removed.

Thus, our primary objective was to determine the effectiveness of heat treatment via aerated steam mixtures, and fumigation with metam sodium, in eradicating *P. ramorum* (the European A1 and North American A2 mating types) and other soilborne pathogens including *Pythium irregulare, Thielaviopsis basicola,* and *Cylindrocladium scoparium* introduced into soil-less potting media or soil. A secondary objective with the heat treatment was to confirm that pathogens could be eliminated in plastic containers without melting the containers.

## Materials and methods

INOCULUM AND MIX PREPARATION. Cultures of *C. scoparium, P. ramorum* isolates D12A and 2027, *P. irregulare,* and *T. basicola* were grown on dilute V8 Juice agar (50 mL•L$^{-1}$ clarified V8 juice; Ribeiro, 1978) for 21 d in a dark incubator at 20 °C. Chlamydospore (oospores for *P. irregulare)* inoculum was prepared by adding 420 mL of clarified V8 broth to 600 cm$^3$ of dry vermiculite contained in a 1.6-L glass jar system using autoclavable/breathable lids and contaminant barrier filters. Jars were then autoclaved twice with an overnight cooling period between treatments. Thirty 6-mm-diameter mycelial plugs of a desired isolate were transferred aseptically from 14-d-old agar culture plates to each glass jar. These were stored in a dark incubator at 20 °C for 2 months. Jars of control inoculum received an equivalent number of sterile agar plugs. Before incorporation of vermiculite inoculum into the potting mix, the inoculum was air-dried for 48 h to a moisture level suitable for easy mixing, and was tested for viability.

AERATED STEAM TREATMENT. Inoculum was manually incorporated into potting mix (OBC #2 bedding mix; OBC Northwest, Canby, OR) at a 10% rate by volume. After rotating and mixing in plastic bags for 5 min to homogenize the contents, inoculated bags were incubated in the dark at 20 °C. Bags were rotated and reshaken at 2-week intervals for 1.5 months. Random samples from each infested mix were then removed and baited to confirm pathogen viability before steam treatments.

Polystyrene plug trays (12 x 24 cells) were filled with inoculated mixes. One row of 12 cells in each tray was filled with each pathogen treatment, using the same row placement in each tray. A blank row was left between each treatment row to prevent cross- contamination between treatments. Aluminum foil was placed over blank rows and filled rows to prevent contamination as new treatment rows were filled. Unfilled spacer rows were used on each end of the tray, as well as two unfilled rows between each treatment row. Three replicate trays containing all pathogens were used for each temperature treatment.

As each treatment row was filled, it was carefully moistened by pipetring water to settle the contents. After an entire tray was filled, it was enclosed in a jacket of aluminum foil. Trays then were incubated at 20 °C for 48 h. The lower foil was left in place as trays were placed into the steam treatment chamber.

A metal chemical storage cabinet was converted into a steam chamber with expanded metal shelves on which to place treatment trays. The chamber was designed to introduce the air-steam mixtures through a manifold system to equalize distribution within the chamber. The manifolds were located at the top and bottom and on both sides of the chamber. Air was mixed with steam from a portable generator in varying proportions to accomplish the desired temperatures before introduction into the chamber. Air and steam inputs were regulated and then mixed to achieve six target temperatures ranging from 45 to 70 °C, inclusive, at 5 °C increments. At each temperature, three replicate trays, placed on different height shelving in the chamber, were air-steam treated for 30 min. Generally, an additional 5 to 7 min was needed to allow the chamber and its contents to equilibrate each medium temperature before timing.

After air-steam treatments, flats were removed and cooled at room temperature for 1 h before beginning recovery assays. If a treatment could not be baited within 2 h of cooling, the flat was put into cold storage at 5 °C.

The study was conducted twice. In the second trial, the potting mix base was changed to a 70:30 (by volume) mix of peatmoss (Lakeland Peat; Sun Gro Horticulture, Hubbard, OR) and horticultural grade perlite (Supreme Perlite, Portland, OR). Data from the two trials were combined for analysis because variance among trials was homogeneous according to Bartlett's test.

METAM SODIUM TREATMENT. Similar infested potting media used in the second aerated-steam treatment experiment, as well as a sandy loam soil, was used for treatments with different rates of metam sodium. The potting medium was placed into 20 x 20-cm resealable polyethylene bags (600 $cm^3$ per bag) and was moistened with 200 mL of water containing metam sodium at rates of 0.25 to 1.0 mL•L' substrate. Metam sodium was applied in the final 10 mL of total fluid. The bags were sealed and incubated in a fume hood for 48 h at room temperature, after which the bags were opened to vent off the MIT released from the metam sodium. Bags were shaken periodically and remained unsealed in the fume hood for 14 d to completely exhaust the MIT. Recovery assays to detect survival of the different pathogens was thereafter the same as for the aerated steam treatments. The metam sodium treatment experiment was conducted twice.

In another experiment using only *P. ramorum,* sporangia were added to previously pasteurized sandy loam soil. The soil was wet up with 90 mL of water in small aliquots to maintain friability and kept moist in plastic zip-lock bags for 1 week. This treatment was meant to simulate the transfer of sporangia from infected plants in containers to the soil beneath the container (Jeffers, 2005) before treating the soil with different concentrations of metam sodium in water. After 48 h of treatment with the different metam sodium rates, treatment bags were opened, vented for 14 d, and the-soil was baited as described below.

RECOVERY ASSAYS. In all studies, each pathogen was baited to determine survivability at individual temperatures or at individual rates of metam sodium. The double-cup leaf disc baiting method (Linderman and Zeitoun, 1977) was used to detect survival of the *P. ramorum* and *P. irregulare* isolates. A 150-mL wax paper cup with its bottom replaced by a double layer of cheesecloth was positioned firmly upon 15 $cm^3$ of sampled substrate contained inside a second intact cup. Camellia *(Camellia sasanqua)* leaves were washed in water and were surface-disinfested by immersion in 0.06% sodium hypochlorite for 10 min and were then allowed to air-dry. Twelve leaf discs (6 mm in diameter) were cut from the leaves and floated on the surface of 50 mL of distilled water in each cup system for 20 h at 20 °C. Ten discs then were retrieved from each cup with forceps, blotted dry on clean paper towels, and plated on PARP medium (containing 10 mg•L pimaricin, 25 mg•$L^{-1}$ ampicillin, 50 mg•$L^{-'}$ rifampicin, and 100 mg•$L^{-1}$ pentachloronitrobenzene; Kannwischer and Mitchell, 1978). Five discs were placed on each of two plates. Plates were incubated in the dark for 5 to 7 d, after which colonies of the pathogens were counted. The percentage of leaf discs colonized out of 10 total discs was calculated for each replicate.

Preliminary trials indicated some variability in consistently baiting P. *ramorum* and *P. irregulare,* even in nontreated controls, probably from uneven distribution of inoculum. However, direct substrate plating of the infested media indicated the viability of the pathogens. A small 1-$cm^3$ subsample from each bagged treatment was sprinkled onto PARP and was incubated along with the bait disc plates.

Recovery of C. *scoparium* was by means of the azalea *(Rhododendron sp.)* leaf trapping method (Linderman, 1972). Five azalea leaves were placed lower side up on moist vermiculite contained in deep plastic petri dishes. A small aliquot of treated potting mix was placed on a leaf. Ten total samples were placed on 10 leaves for each treatment replicate. Leaves were incubated at 20 °C for 1 week, after which the percentage of leaves showing lesions was calculated for each replicate. That the lesions were caused by *C. scoparium* was confirmed by examining sporulation on the lesions or by tissue plating and observing sporulation on recovered colonies.

*T. basicola* was baited using carrot *(Daucus carota)* discs (Yarwood, 1946). Fresh carrots were scrubbed vigorously and rinsed clean with distilled water to remove epidermal cells and debris. A vegetable slicer was used to make thin, cross-section discs. Five discs (3 mm thick) were placed on moist vermiculite contained in deep petri dishes. Sampling and incubation conditions were the same as for *C. scoparium.* The presence of sporulation (endoconidia and chlamydospores) was used to confirm that *T. basicola* was the pathogen colonizing the carrot discs.

Statistical comparisons between pathogens were not designed because of the inherent variability in propagule formation in inocula or modes of recovery. Untransformed means with 95% confidence intervals are reported in tables. Data from direct plating of substrates are reported as the number of positive recovery plates of three replicates per treatment.

## Results and discussion

AERATED STEAM TREATMENTS. Treatment of infested potting media and plastic containers with different temperatures established in air-steam mixtures clearly showed that

**Table 1. Recovery of *Cylindrocladium scoparium*, *Phytophthora ramorum*, *Pythium irregulare*, and *Thielaviopsis basicola* from infested soil-less potting mix treated with aerated steam.**

| | Recovery from baits [mean ± 95% confidence interval (%)][z] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Temperature treatments (°C)[y] | | | | | | |
| Pathogen | Unheated | 45 | 50 | 55 | 60 | 65 | 70 |
| *C. scoparium* | 97 ± 4 | 56 ± 22 | 0 | 0 | 0 | 0 | 0 |
| *P. ramorum* D12A[x] | 77 ± 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. ramorum* 2027[x] | 85 ± 14 | 7 ± 7 | 0 | 0 | 0 | 0 | 0 |
| *P. irregulare* | 98 ± 3 | 23 ± 10 | 0 | 0 | 0 | 0 | 0 |
| *T. basicola* | 98 ± 3 | 63 ± 30 | 0 | 0 | 0 | 0 | 0 |

[z]Percentage of sasanqua camellia leaf disc baits from which *P. ramorum* and *P. irregulare* grew on PARP selective medium, the percentage of azalea leaves from which *C. scoparium* grew, or the percentage of carrot discs from which *T. basicola* grew. PARP medium contained 10 mg·$L^{-1}$ (ppm) pimaricin, 250 mg·$L^{-1}$ ampicillin, 50 mg·$L^{-1}$ rifampicin, and 100 mg·$L^{-1}$ pentachloronitrobenzene added to cornmeal agar (Kannwischer and Mitchell, 1978). Percentages were calculated from the number of baits positive of 10 in each replicate, three replicates per treatment ± 95% confidence intervals. Data are combined results of two trials that demonstrated overall homogeneity of variance by Bartlett's test.

[y]Temperature fluctuation over each 30-min treatment period was ± 2 °C; (°C × 1.8) + 32 = °F.

[x]*P. ramorum* mating types: D12A = European A1; 2027 = North American A2.

Table 2. Recovery via bait discs (B) or direct plating (DP) of *Cylindrocladium scoparium*, *Phytophthora ramorum*, *Pythium irregulare*, and *Thielaviopsis basicola* from infested potting mix or soil treated with metam sodium.

| | Metam sodium treatments (mL·L$^{-1}$ potting medium)[y] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | None | | 0.25 | | 0.50 | | 1.00 | |
| | Recovery (mean ± 95% confidence interval) from B (%)[x] or DP (no. plates)[w] | | | | | | | |
| Substrate[z]/pathogen | B | DP | B | DP | B | DP | B | DP |
| Potting mix | | | | | | | | |
| *C. scoparium* | 93 ± 7 | na[v] | 0 | na | 0 | na | 0 | na |
| *P. ramorum* D12A[u] | 57 ± 7 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. ramorum* 2027[u] | 57 ± 17 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. irregulare* | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *T. basicola* | 100 | na | 97 ± 7 | na | 20 ± 11 | na | 0 | na |
| Soil | | | | | | | | |
| *C. scoparium* | 73 ± 13 | na | 27 ± 7 | na | 13 ± 7 | na | 0 | na |
| *P. ramorum* D12A | 100 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. ramorum* 2027 | 100 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. irregulare* | 21 | 3 | 17 | 3 | 0 | 3 | 0 | 0 |
| *T. basicola* | 100 | na | 93 ± 13 | na | 53 ± 13 | na | 0 | na |

[z]Substrates: potting mix of 70:30 (by volume) peatmoss:perlite or alluvial sandy loam soil.
[y]1 mL·L$^{-1}$ = 0.9575 fl oz/ft$^3$.
[x]Percentage of sasanqua camellia leaf disc baits from which *P. ramorum* and *P. irregulare* grew on PARP selective medium, the percentage of azalea leaves from which *C. scoparium* grew, or the percentage of carrot discs from which *T. basicola* grew. Percentages were calculated from the number of baits positive of 10 in each replicate, three replicates per treatment ± 95% confidence intervals. PARP medium contained 10 mg·L$^{-1}$ (ppm) pimaricin, 250 mg·L$^{-1}$ ampicillin, 50 mg·L$^{-1}$ rifampicin, and 100 mg·L$^{-1}$ pentachloronitrobenzene added to cornmeal agar (Kannwischer and Mitchell, 1978).
[w]Supplemental direct plating for detection of pathogen when camellia disc baits yielded no recovery for *P. ramorum* or *P. irregulare*. The number of plates from which the same species were recovered by direct plating a 1-cm$^3$ (0.06-inch$^3$) subsample on PARP-selective medium. Direct plate numbers are given as the number of positive detection plates of three replicate plates per treatment.
[v]DP not applicable to this pathogen.
[u]*P. ramorum* mating types: D12A = European A1; 2027 = North American A2.

temperatures of 50 °C or higher for 30 min eliminated all pathogens tested without melting the plastic plug flats (Table 1). These results are in keeping with earlier guidelines on the use of aerated steam presented by Baker (1957), except that here we report effects on *P. ramorum* that only recently was shown to be an important nursery pathogen that can survive in potting media (Linderman and Davis, 2006). Other studies have demonstrated the utility of composting heat to eradicate *P. ramorum* (Swain et al., 2006), but Harnik et al. (2004) had difficulty in killing the pathogen in dry California bay laurel (*Umbellularia californica*) leaves, used as a spice, and a combination of dry heat and vacuum were needed to kill the pathogen. Possibly, the pathogen was present as chlamydospores in the dry leaves and the use of dry heat accounted for the difficulty encountered.

Regarding sanitization of containers, those used in earlier years were wooden flats and clay pots that could tolerate higher temperatures, whereas currently plastic containers are used. This study documents the utility of aerated steam treatments to sanitize plastic containers that might be contaminated with pathogens from previous infected plants grown in them. Personal observations (R.G. Linderman) in recent years have shown that the root rot pathogens *T basicola* and *Pythium spp.* can survive in used plug flats, even after pressure washing and drying. Personal communication with growers (R.G. Linderman) indicates that aerated steam treatments in simple plywood chambers appear to be effective in eradicating pathogens from the containers. Thus, aerated steam treatments in inexpensively built chambers can effectively replace pot and plug flat chemical or hot water dip tanks, with assurance that the containers have been sanitized and are free of pathogens.

METAM SODIUM TREATMENTS. Treatment of pathogen-infested potting medium or soil with metam sodium at 1.0 mL•L---$^1$ of medium was effective in eradicating all the soilborne pathogens tested (Table 2). All pathogens were eliminated at the highest rate, and isolate sensitivity was apparent. *P. ramorum* was effectively killed at the lowest rate, but *C. scoparium* and *T. basicola* were recovered at the intermediate rates (0.25 and 0.50 mL•L$^{-1}$) and were killed at the high rate (1.0 mL•L'). Metam sodium has been available as a soil fumigant for many years, and is known to be effective in eradicating soilborne pathogens and weeds (Munnecke and van Gundy, 1979). However, we have demonstrated that it can be an effective treatment in eradicating *P. ramorum* from contaminated potting medium. Thus, growers would have a means of treating potting media to eradicate *P. ramorum* and any other soilborne pathogens, as well as treating *P. ramorum*-contaminated soil from beneath containers with infected plants. Metam sodium is available in liquid form to be applied as a drench or mixed in as a granular form as a means of releasing MIT as the toxic fumigant. The liquid form would be the best method for treating soil beneath pots where cultivation and incorporation of the granular form is not possible.

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