Survival of *Phytophthora ramorum* Compared to Other Species of *Phytophthora* in Potting Media Components, Compost, and Soil

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ADDITIONAL INDEX WORDS. **ornamentals, *Phytophthora cactorum, P. citricola, P. citrophthora*, sudden oak death, ramorum blight, ramorum shoot dieback**

SUMMARY. ***Phytophthora ramorum*, while thought to be primarily an aboveground pathogen, can be introduced into soilless potting media in the nursery industry as sporangia or chlamydospores and remain undetected while disseminated geographically. Inoculum of this pathogen, both North American (A-2 mating type) and European (A-1 mating type) isolates, was used to infest potting media components or soil, using either sporangia, chlamydospores produced in vermiculite culture, or dry infected 'Nova Zembla' rhododendron (*Rhododendron* sp.) leaf pieces. Vermiculite chlamydospore/oospore inoculum of *P. citricola, P. cactorum,* and *P. citrophthora* were included for comparison. Survival was determined monthly by leaf disc baiting or direct plating on selective medium. Results indicated that *P. ramorum* survived in most media components or soil for up to 6 months when introduced as sporangia, or up to 12 months as chlamydospores. However, it was not detected at all from infected rhododendron leaf pieces by either detection method. These results show that *P. ramorum* can survive in potting media if introduced as sporangia or chlamydospores, and accordingly the pathogen could be disseminated geographically without being detected visually.**

The discovery of the ramorum blight and shoot dieback pathogen, *Phytophthora ramorum*, killing trees and shrubs in the forests of California and Oregon, as well as ornamental plants in U.S. nurseries and nurseries and landscapes in several European countries, underscores the threat that this pathogen poses to nurseries growing susceptible plants, especially those in the Ericaceae, such as rhododendron (*Rhododendron* spp.); Fagaceae, such as oaks (*Quercus* spp.); Caprifoliaceae, such as viburnum (*Viburnum* spp.); and Theaceae, such as camellia (*Camellia* spp.) (Davidson et al., 2003; Goheen et al., 2002; Parke et al., 2004; Rizzo et al., 2002; Werres et al., 2001). However, Linderman et al. (2006) demonstrated that there are many other potential host species that are susceptible in artificial inoculations that have not been reported as being naturally infected. Furthermore, they showed that foliar symptoms caused by *P. ramorum* are indistinguishable from those that could be caused by other species of *Phytophthora*. In addition, there is an extensive list of host plants from which *P. ramorum* has been isolated but Koch's postulates have not been completed to prove pathogenicity (associated hosts). *P. ramorum* is considered to be largely a foliar pathogen on nursery crops (Werres et al., 2001). However, it could be incorporated as sporangia, zoospores, or chlamydospores from aboveground infections into container soilless potting media and thus become soilborne. If that were to happen, the pathogen may remain undetected by visual plant symptoms and, thus, be disseminated geographically.

Thus, our objective was to determine the capacity of *P. ramorum* (both A1 and A2 mating types) to survive in potting media components or soil, and to compare survival of *P. ramorum* with other well-known soilborne pathogens, including *P. cactorum*, *P. citricola*, and *P. citrophthora*, that also cause phytophthora blight and dieback in nursery crops and other hosts as well (Coyier and Rhone, 1986; Osterbauer et al., 2004). A preliminary report has been published (Linderman and Davis, 2005).

Materials and methods

All experiments were conducted at the U.S. Department of Agriculture, Agricultural Research Service Horticultural Crops Research Laboratory in Corvallis, Ore., under appropriate permits and quarantine conditions required and approved by the Oregon Department of Agriculture, and only with pathogens isolated in Oregon.

EXPERIMENTAL TREATMENTS. Eight potting mix components were infested separately with three forms of inoculum of each of five *Phytophthora* isolates or species, plus a non-infested control. Potting mix components included: peatmoss, douglas-fir bark, redwood sawdust, coconut fiber dust (coir), alluvial sand, garden clay loam soil, a custom soilless mix (1 douglas-fir bark : 1 peatmoss : 1 pumice), and a dairy manure-based compost. *Phytophthora cactorum, P. citricola*, *P. citrophthora*, and two isolates of *P. ramorum* were used (sources are listed in Table 1). The three forms of inoculum used were chlamydospores in vermiculite, dried infected 'Nova Zembla' rhododendron leaf pieces, and sporangia washed from culture plates. There were three replications per treatment combination of potting

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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
29.5735	fl oz	mL	0.0338
7.8125	fl oz/gal	$mL \cdot L^{-1}$	0.1280
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	$mg \cdot L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

Table 1. *Phytophthora* species and isolates from Oregon used in studies comparing survival in soilless potting media or soil.

Phytophthora species (isolate no.)	Collector	Host of origin
P. cactorum (25-4-3)	P. Reeser (Oregon State University)	Rhododendron (*Rhododendron* sp.)
P. citricola (Pc 98-517)	P. Reeser (Oregon State University)	Rhododendron
P. citrophthora (Pc 01-02)	P. Reeser (Oregon State University)	Rhododendron
P. ramorum (03-74-D12A) (European A1 mating type)	N. Osterbauer (Oregon Dept. of Agriculture)	Doublefile viburnum (*Viburnum plicatum* var. *tomentosum* 'Mariesii')
P. ramorum (2027) (North American A2 mating type)	E. Hansen (Oregon State University)	Tanoak (*Lithocarpus densiflorus*)

media component × inoculum form × *Phytophthora* isolate. Pathogen survival was examined monthly by means of leaf disc trapping (Linderman and Zeitoun, 1977) or direct plating on PARP selective medium [containing 10 mg·L^{-1} pimaricin, 250 mg·L^{-1} ampicillin, 50 mg·L^{-1} rifampicin, and 100 mg·L^{-1} pentachloronitrobenzene (Kannwischer and Mitchell, 1978)]. The soil substrate treatment contained *Pythium*, requiring the addition of 25 mg·L^{-1} hymexazol to the PARP medium (PARPH) when isolating from that substrate.

All potting component media were pre-moistened before adding inoculum to minimize disturbance and reduce contamination. Infested potting media were stored in 20 × 20-cm resealable polyethylene bags. After sealing, each bag was manually rotated and shaken for 1.5 min to homogenize the contents. Bags were reshaken at 2-week intervals and all treatments were incubated in the dark at 20 °C.

Infected leaf inoculum preparation and application. 'Nova Zembla' rhododendron leaves that previously had been inoculated with each of the five *Phytophthora* isolates and become fully colonized after 3–4 weeks, were used as an inoculum source and assayed for survival. Microscopic examination of cleared leaves revealed that abundant chlamydospores of *P. ramorum*, relatively few chlamydospores of *P. citrophthora*, and fairly abundant oospores of *P. cactorum* and *P. citricola* were present in the tissue. The infected leaves were air-dried for 2 months in paper bags, then manually crumpled into particulates smaller than 2 cm. Thirty cubic centimeters of crumpled leaves was added to 450 cm^3 of pre-moistened potting medium contained in a polyethylene bag. Bags were sealed, shaken, and incubated as above.

Sporangia inoculum. Fourteen-day-old cultures of only *P. ramorum* grown on dilute V8 juice agar were used to collect sporangial inoculum to be added to the potting mix components. Culture plates were flooded with 3 mL of sterile distilled water, and the surface of the agar scraped with the edge of a spatula to remove the cauducous sporangia, collected into beakers to which more sterile water was added to increase volume. Each suspension of sporangia was continually stirred while 8-mL aliquots were pipetted into each bag of pre-moistened potting mix component. Bags were sealed, shaken, and incubated.

Vermiculite inoculum preparation and application. All *Phytophthora* isolates were grown on dilute V8 juice agar (30 mL·L^{-1} clarified V8 juice) (Linderman et al., 2006) in petri dishes for 14 d in a dark incubator at 20 °C. Chlamydospore inoculum was prepared by adding 420 mL clarified V8 broth (Ribeiro, 1978) to 600 cm^3 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 sterilizations. Thirty 6-mm-diameter mycelial agar plugs of a desired isolate were transferred aseptically from 14-d-old 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. Prior to incorporation of vermiculite inoculum into media components, the inoculum was placed in cheesecloth and washed with water to remove excess nutrients and culture metabolites, and air-dried for 48 h to a moisture level suitable for easy mixing.

For vermiculite-chlamydospore inoculum treatments, 50 cm^3 of air-dried inoculum was added to 450 cm^3 of potting medium contained in each bag. Bags were sealed, shaken, and incubated as above.

Recovery assays. Each *Phytophthora* isolate × media component treatment for each inoculum form was baited monthly to monitor survival and recovery. The double-cup leaf disc baiting (B) method (Linderman and Zeitoun, 1977) was used to detect survival of the *Phytophthora* 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. Sasanqua camellia (*C. sasanqua*) leaves were washed in water and surface-disinfested by immersion in 0.06% sodium hypochlorite for 10 min, and allowed to air-dry. Five leaf discs (6-mm-diameter) were cut from the leaves and floated on the surface of 50 mL of distilled water in each cup system for 24 h at 20 °C. Discs then were retrieved from each cup with forceps, blotted dry on clean paper towels, and plated on PARP medium (Kannwischer and Mitchell, 1978). A small 1-cm^3 subsample from each bagged treatment was also sprinkled onto PARP or PARPH for direct plating (DP) without baiting. Plates were incubated in the dark for 5–7 d, after which colonies were counted, then stored and observed frequently for the presence of chlamydospores, sporangia, or oospores as final confirmation of species identity. The percentage of leaf discs colonized by the isolates was calculated separately for each replicate sample. Direct substrate plating was rated as plus or minus for the presence or absence of the pathogen.

While in the process of sampling for recovery, each bag of potting substrate was re-wetted with distilled water using a hand mister.

Data compilation and analysis. Percent recovery data from baited leaf discs were transformed to arcsine-square root values prior to analysis of variance. Data were analyzed as a repeated-measure design using Systat (version 8.0; SPSS, Inc., Evanston, Ill.). Month was the repeated variable (not a regressor); media component and *Phytophthora* isolate were the fixed effects. Statistical comparisons between *Phytophthora* isolates were not designed because of the inherent variability in infective propagule formation in the inocula. 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 out of three replicates per treatment.

Results

In each recovery study significant differences ($P \leq 0.001$) existed among the main effects of media components, *Phytophthora* isolates, and time. Significant interactions ($P \leq 0.001$) with time also were indicated, supporting our observations that changes in recovery over time were not the same for all components or isolates.

INFECTED LEAF INOCULUM. Rhododendron leaf pieces infected with *P. cactorum, P. citricola, P. citrophthora*, and *P. ramorum* (both isolates 2027 and D12A) were used as inoculum to infest media components or soil. *P. ramorum* and *P. citrophthora* were never recovered by either B or DP methods at any time up to 14 months (Table 2). By comparison, *P. cactorum* was detected in most substrates, by either B or DP, for up to 6 months, but not 14 months. *P. citricola* was recovered erratically from different media components, ranging from 0 (sawdust) to 3 (coir, bark, peatmoss, and potting mix) months; it survived best in sand or soil (5 months).

SPORANGIAL INOCULUM. Media components were only infested with sporangial inoculum of *P. ramorum* because of the production of abundant sporangia in culture compared to the other species. Both isolates were detected for up to 6 months by B or DP methods from all substrates amended (Table 3). Survival varied depending on the component substrate, with the best (ranging from 4–6 months) being in coir, compost, bark, peatmoss, and potting mix, and the poorest (1–6 months) in sand, soil, or sawdust. Survival was generally better with isolate D12A (A-1 mating type) than 2027 (A-2 mating type), and detection by B was less than by DP, suggesting reduced production of sporangia and zoospores in the baiting system. That was especially true in sand, soil, or sawdust where recovery by B was only 1–3 months for either isolate.

VERMICULITE CHLAMYDOSPORE/OOSPORE INOCULUM. Vermiculite culture of the different species of *Phytophthora* resulted in the formation of abundant chlamydospores by *P. ramorum*, few chlamydospores by *P. citrophthora*, and moderate numbers of

Table 2. Recovery of *Phytophthora cactorum* or *P. citricola* from potting media components infested with air-dried infected 'Nova Zembla' rhododendron leaf pieces, using sasanqua camellia leaf disc baiting or direct plating on PARP selective medium.[z]

	Time after inoculation (months)													
	1		2		3		4		5		6		14	
Media component[y]/	Recovery from leaf bait discs [B (%)][x] or direct plating [DP (no. plates)][w]													
Phytophthora isolate	B	DP	B	DP	B	DP	B	DP	B	DP	B	DP	B	DP
Coir dust														
P. cactorum	0	1	0	1	27 ± 13	3	7 ± 13	1	7 ± 13	2	27 ± 13	0	0	0
P. citricola	0	0	7 ± 13	0	13 ± 13	2	0	0	0	0	0	1	0	0
Compost														
P. cactorum	0	0	13 ± 13	3	0	1	7 ± 13	3	47 ± 35	3	0	2	0	0
P. citricola	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Fir bark														
P. cactorum	40 ± 23	3	40 ± 39	2	33 ± 13	3	20 ± 0	2	67 ± 35	2	60 ± 23	2	0	0
P. citricola	0	3	0	1	0	1	0	0	0	0	0	0	0	0
Peatmoss														
P. cactorum	0	2	0	2	0	1	0	0	0	3	0	1	0	0
P. citricola	0	2	0	0	0	0	0	0	0	0	0	1	0	0
Potting mix														
P. cactorum	27 ± 13	2	60 ± 23	2	53 ± 13	3	53 ± 34	2	73 ± 16	3	53 ± 13	3	7 ± 13	1
P. citricola	7 ± 13	1	20 ± 39	2	27 ± 13	3	0	1	0	0	0	0	0	0
Sand														
P. cactorum	7 ± 13	0	33 ± 27	3	47 ± 13	3	33 ± 26	1	73 ± 13	1	87 ± 13	2	0	0
P. citricola	0	0	47 ± 26	2	33 ± 13	3	27 ± 13	0	20 ± 23	0	0	0	0	0
Sawdust														
P. cactorum	0	2	7 ± 13	3	33 ± 26	3	27 ± 13	2	20 ± 23	2	0	1	0	0
P. citricola	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Soil														
P. cactorum	0	1	73 ± 13	3	47 ± 13	3	13 ± 27	1	7 ± 13	2	0	0	0	0
P. citricola	0	0	60 ± 0	3	33 ± 13	3	33 ±27	2	7 ±13	0	0	0	0	0

[z]Contains 10 mg·L^{-1} (ppm) pimaricin, 250 mg·L^{-1} ampicillin, 50 mg·L^{-1} rifampicin, and 100 mg·L^{-1} pentachloronitrobenzene (Kannwischer and Mitchell, 1978).
[y]Potting media components: coir dust = coconut husk fiber, compost = dairy manure base, fir bark = douglas fir fine grade bark, potting mix = 40 peatmoss : 30 fine douglas fir bark : 30 pumice (% by volume), sand = alluvial base, sawdust = redwood sawdust, soil = silty clay loam.
[x]Number of plates from which the same species were recovered by direct plating a 1-cm^3 (0.06 inch^3) sub-sample on PARP selective medium. Direct plate numbers are given as the number of positive detection plates out of three replicate plates per treatment.
[w]Percentage of sasanqua camellia leaf disc baits from which *Phytophthora* species grew on PARP selective medium or PARPH medium (PARP with 25 mg·L^{-1} hymexazol). Percentages were calculated from the number of baits positive out of five in each replicate, three replicates per treatment ± 95% confidence intervals.

Table 3. Recovery of *Phytophthora ramorum* isolates from potting media components infested with *P. ramorum* sporangia, using sasanqua camellia leaf disc baiting or direct plating on PARP selective medium.[z]

Media component[y]/ *Phytophthora* isolate	Time after inoculation (months) 1		2		3		4		5		6		14	
	Recovery from leaf bait discs [B (%)][x] or direct plating [DP (no. plates)][w]													
	B	DP	B	DP	B	DP	B	DP	B	DP	B	DP	B	DP
Coir dust														
P. ramorum 2027	73 ± 13	3	60 ± 23	3	7 ± 13	1	7 ± 13	1	7 ± 13	2	0	0	0	0
P. ramorum D12A	93 ± 13	3	40 ± 27	3	33 ± 13	3	47 ± 13	2	53 ± 13	3	0	2	0	0
Compost														
P. ramorum 2027	0	0	0	0	0	0	0	0	0	1	0	0	0	0
P. ramorum D12A	27 ± 26	3	7 ± 13	2	0	0	0	1	0	1	0	1	0	0
Fir bark														
P. ramorum 2027	27 ± 26	0	0	3	0	0	7 ± 13	0	0	0	0	0	0	0
P. ramorum D12A	67 ± 35	3	33 ± 13	3	33 ± 13	1	33 ± 13	2	33 ± 13	2	33 ± 35	3	0	0
Peatmoss														
P. ramorum 2027	67 ± 13	3	0	2	20 ± 0	3	0	0	0	1	0	0	0	0
P. ramorum D12A	100	3	67 ± 13	3	53 ± 26	3	13 ± 13	2	13 ± 8	2	0	0	0	0
Potting mix														
P. ramorum 2027	100	3	73 ± 26	1	33 ± 13	3	7 ± 13	0	13+13	0	47 ± 26	0	0	0
P. ramorum D12A	100	3	100 ± 0	3	73 ± 16	3	67 ± 8	2	60+14	3	20 ± 14	1	0	0
Sand														
P. ramorum 2027	27 ± 26	2	0	0	0	0	0	0	0	0	0	0	0	0
P. ramorum D12A	93 ± 13	3	60 ± 23	3	0	0	0	0	0	0	0	0	0	0
Sawdust														
P. ramorum 2027	47 ± 26	2	0	1	0	3	0	2	0	1	0	0	0	0
P. ramorum D12A	67 ± 26	3	0	3	0	2	0	3	0	3	0	3	0	0
Soil														
P. ramorum 2027	67 ± 13	3	53 ± 13	3	0	0	0	0	0	0	0	0	0	0
P. ramorum D12A	60 ± 0	3	67 ± 13	3	67 ± 13	3	0	1	0	0	0	0	0	0

[z]Contains 10 $mg \cdot L^{-1}$ (ppm) pimaricin, 250 $mg \cdot L^{-1}$ ampicillin, 50 $mg \cdot L^{-1}$ rifampicin, and 100 $mg \cdot L^{-1}$ pentachloronitrobenzene (Kannwischer and Mitchell, 1978).
[y]Potting media components: coir dust = coconut husk fiber, compost = dairy manure base, fir bark = douglas fir fine grade bark, potting mix = 40 peatmoss:30 fine douglas fir bark:30 pumice (% by volume), sand = alluvial base, sawdust = redwood sawdust, soil = silty clay loam.
[x]Number of plates from which the same species were recovered by direct plating a 1-cm^3 (0.06 $inch^3$) sub-sample on PARP selective medium. Direct plate numbers are given as the number of positive detection plates out of three replicate plates per treatment.
[w]Percentage of sasanqua camellia leaf disc baits from which *Phytophthora ramorum* grew on PARP selective medium or PARPH medium (PARP with 25 $mg \cdot L^{-1}$ hymexazol). Percentages were calculated from the number of baits positive out of five in each replicate, three replicates per treatment ± 95% confidence intervals.

oospores by *P. cactorum* and *P. citricola* (based on direct microscopic observations). For *P. cactorum*, *P. citricola*, and *P. citrophthora*, survival varied with the substrate and detection method: coir, 2–4 months; compost, 0–3 months except for *P. cactorum* at 6–12 months; bark, 2–4 months; peatmoss 2–4 months with reduced recovery by B; potting mix, 1–4 months; sand, 1–6 months; sawdust, 1–4 months; and soil, 1–5 months with most being only 1 month (Table 4). In contrast, *P. ramorum* (both isolates) generally survived 12 months, except for sand substrate at 5–6 months.

Discussion

This study has demonstrated the capacity of *P. ramorum* to survive in potting mix components, compost, or soil, starting from infestation with sporangia or chlamydospores from culture. By comparison, the two isolates of *P. ramorum* survived in potting mix components as well as other soilborne pathogens, perhaps even better in that their capacity to produce resting spores (chlamydospores or oospores) may be greater than that of the other species tested. However, since we did not quantify the inoculum before infestation, differences in recovery could be related to inoculum density. The implication from this study is that if inoculum produced from aboveground infections were introduced into the potting mix, whether as sporangia or chlamydospores, the fungus could survive for extended periods in the medium without being detected. By this means it could be disseminated to other geographic locations. The potential of the pathogen in the medium to initiate disease at that location remains to be demonstrated. However, it has been shown that *P. ramorum* can infect roots and the pathogen can move in the vascular system up into the aboveground portions of the plant (Lewis et al., 2004; Shishkoff and Tooley, 2004).

The lack of recovery of *P. ramorum* from infected rhododendron leaves that are known to contain abundant chlamydospores (our observations; Shishkoff and Tooley, 2004; Tooley et al., 2004) remains unexplained. We were able to isolate or trap *P. cactorum* successfully from parallel treatments (presumably surviving as oospores), so we do not know why we did not recover *P. ramorum* from parallel treatments. Perhaps viable chlamydospores were embedded in host plant tissue but were not able to germinate on PARP selective medium or produce sporangia and zoospores that would be trapped by baiting. Schishkoff and Tooley (2004) were able to remove chlamydospores from infected plant tissue buried in container medium and demonstrate their viability by plating onto selective medium. However, the infected leaf tissue was not allowed to

Table 4. Recovery of *Phytophthora* species from potting media components infested with vermiculite cultures containing chlamydospores or oospores, using sasanqua camellia leaf disc baiting or direct plating on PARP selective medium.[z]

Media component[y]/	Time after inoculation (months)													
	1		2		3		4		5		6		14	
	Recovery from leaf bait discs [B (%)][x] or direct plating [DP (no. plates)][w]													
Phytophthora isolate	B	DP	B	DP	B	DP	B	DP	B	DP	B	DP	B	DP
Coir dust														
P. cactorum 2027	87 ± 13	2	53 ± 13	3	20 ± 23	2	0	0	0	0	0	0	0	0
P. citricola	33 ± 13	3	7 ± 13	3	0	0	0	1	0	0	0	0	0	0
P. citrophthora	73 ± 35	2	27 ± 13	1	0	1	0	0	0	0	0	0	0	0
P. ramorum 2027	100	3	80 ± 0	3	93 ± 13	3	73 ± 35	3	66 ± 35	3	93 ± 13	3	33 ± 13	2
P. ramorum D12A	100	3	93 ± 13	3	100	1	100	3	100	3	100	2	53 ± 26	0
Compost														
P. cactorum	60 ± 23	2	13 ± 13	3	13 ± 13	0	13 ± 26	2	7 ± 13	3	13 ± 26	3	0	3
P. citricola	0	2	0	3	0	1	0	0	0	0	0	0	0	0
P. citrophthora	53 ± 26	2	13 ± 13	1	0	0	0	0	0	0	0	0	0	0
P. ramorum 2027	53 ± 26	3	27 ± 13	1	0	3	0	3	13 ± 26	2	87 ± 13	3	20	3
P. ramorum D12A	53 ± 26	2	0	2	0	2	33 ± 35	2	27 ± 32	3	40 ± 45	3	53 ± 35	3
Fir bark														
P. cactorum	13 ± 13	3	20 ± 23	1	7 ± 13	0	0	1	0	0	0	0	0	0
P. citricola	0	3	0	2	0	1	0	0	0	0	0	0	0	0
P. citrophthora	47 ± 26	3	27 ± 13	1	0	3	0	1	0	0	0	0	0	0
P. ramorum 2027	87 ± 13	3	60 ± 23	3	20 ± 23	3	13 ± 26	3	40	3	7 ± 13	3	0	1
P. ramorum D12A	100	3	100	3	100	3	93 ± 13	3	93 ± 13	3	100	3	26 ± 26	2
Peatmoss														
P. cactorum	0	3	0	3	0	1	0	0	0	0	0	0	0	0
P. citricola	0	3	0	3	0	0	0	1	0	0	0	0	0	0
P. citrophthora	0	3	0	2	0	1	0	0	0	0	0	0	0	0
P. ramorum 2027	80 ± 0	3	7 ± 13	3	7 ± 13	3	13 ± 26	3	0	2	0	3	20 ± 39	2
P. ramorum D12A	100	3	87 ± 26	3	87 ± 13	2	80	0	93 ± 13	3	60	2	60 ± 22	3
Potting mix														
P. cactorum	67 ± 13	3	7 ± 13	2	0	3	0	0	0	0	0	0	0	0
P. citricola	33 ± 26	3	0	2	0	1	0	1	0	0	0	0	0	0
P. citrophthora	73±13	3	20±0	1	0	0	0	0	0	0	0	0	0	0
P. ramorum 2027	100	3	87±13	3	100	3	100	3	100	3	93±13	3	100	3
P. ramorum D12A	100	2	93±13	3	93±13	3	100	3	100	3	100	3	86±13	2
Sand														
P. cactorum	73±26	2	13±13	2	0	0	20±39	1	0	0	0	0	0	0
P. citricola	67±13	3	7±13	3	0	0	0	0	7±13	0	20±13	1	0	0
P. citrophthora	80±0	3	0	0	0	0	0	0	0	0	0	0	0	0
P. ramorum 2027	100	3	100	2	100	3	87±13	0	100	3	33±13	0	0	0
P. ramorum D12A	10	3	100	3	100	2	100	1	100	3	87±27	3	0	0
Sawdust														
P. cactorum	53±26	3	7±13	3	0	1	0	2	0	0	0	0	0	0
P. citricola	7±13	3	0	3	0	1	0	0	0	0	0	0	0	0
P. citrophthora	40±23	3	53±13	3	33±35	2	0	1	0	0	0	0	0	0
P. ramorum 2027	93±13	2	100	3	73±26	1	87±13	3	93±13	3	100	3	13±13	3
P. ramorum D12A	80±22	2	100	3	100	1	93±13	3	100	3	100	3	100	3
Soil														
P. cactorum	13±13	1	0	0	0	0	0	0	0	1	0	0	0	0
P. citricola	20±0	2	0	0	0	0	0	0	0	0	0	0	0	0
P. citrophthora	40±23	3	0	0	0	0	0	0	0	0	0	0	0	0
P. ramorum 2027	100	3	87±13	3	100	3	33±47	3	87±26	3	80±23	3	13±13	2
P. ramorum D12A	100	3	100	3	100	2	67±13	3	100	3	100	3	40±22	1

[z]Contains 10 mg·L^{-1} (ppm) pimaricin, 250 mg·L^{-1} ampicillin, 50 mg·L^{-1} rifampicin, and 100 mg·L^{-1} pentachloronitrobenzene (Kannwischer and Mitchell, 1978).

[y]Potting media components: coir dust = coconut husk fiber, compost = dairy manure base, fir bark = douglas fir fine grade bark, potting mix = 40 peatmoss : 30 fine douglas fir bark:30 pumice (% by volume), sand = alluvial base, sawdust = redwood sawdust, soil = silty clay loam.

[x]Number of plates from which the same species were recovered by direct plating a 1-cm^3 (0.06 inch^3) sub-sample on PARP selective medium. Direct plate numbers are given as the number of positive detection plates out of three replicate plates per treatment.

[w]Percentage (%) of sasanqua camellia leaf disc baits from which *Phytophthora* species grew on PARP selective medium or PARPH medium (PARP with 25 mg·L^{-1} hymexazol). Percentages were calculated from the number of baits positive out of five in each replicate, three replicates per treatment ± 95% confidence intervals.

dry prior to burial, and apparently was not in direct contact with the medium. How those two factors might have affected their results compared to ours remains unknown. We did not attempt to determine the viability of chlamydospores of *P. ramorum* in the infected, dried rhododendron leaf material, however. Davidson et al. (2005) were not able to recover *P. ramorum* from soil or litter during summer drought conditions when soil moisture content was less than 15%. Similarly, Mircetich and Zentmyer (1966) reported that *P. cinnamomi* may not be able to survive in soil or roots maintained dry at a 3% moisture level. Another possible explanation could be that the chlamydospores did not germinate because of inhibitors of host or microbial origin prior to their release as the host tissue decomposed. Our data suggest that these factors might be operating in some component substrates or soil where recovery from sporangia or cultured chlamydospores was reduced. In addition, in other unpublished work, we have shown that *P. ramorum* is highly sensitive to *in vitro* antagonism by bacterial agents.

Sporangia added to potting mix components survived for up to 6 months, but we do not know by what mechanism that happened. Six months should have exceeded the survival longevity of sporangia unless they converted to some more resistant form with thickened walls. During that period, they also may have germinated and grown vegetatively, possibly producing chlamydospores that accounted for the longer survival period. We did not attempt to make any direct observations of the fungi in the media.

The fact that *P. ramorum* can survive in potting medium and infect roots suggests that containers can also be contaminated, requiring some sanitizing treatment. Studies are in progress to evaluate eradication of the pathogen from infested media and contaminated containers using aerated steam (Baker, 1957; Linderman and Davis, 2005) and fumigation.

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