Recovery of *Phytophthora ramorum* Following Exposure to Temperature Extremes

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ABSTRACT

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We examined the impact of exposure to high and low temperature extremes on recovery of *Phytophthora ramorum* both as free chlamydospores and within infected rhododendron tissue over a 7-day period. Chlamydospores held in moistened sand were incubated at 30, 35, 40, 0, –10, and –20°C for up to 7 days. Infected *Rhododendron* ‘Cunningham’s White’ leaf disks held in sandy loam, loam, or sand at two different soil moisture levels also were subjected to these temperatures for up to 7 days, and to a variable temperature regimen for 12 weeks. Recovery was characterized by growth of *P. ramorum* on selective agar medium following exposures to temperature treatments. Chlamydospores held in moistened sand showed a high rate of recovery at 30°C, steadily declining recovery at 35°C, and no recovery at 40°C over the 7-day period. Chlamydospores were recovered from 0°C after 7 days, with little or no recovery observed at –10 or –20°C. In infected rhododendron tissue, *P. ramorum* was recovered at 20 and 30°C after 7 days but, at 35°C, the pathogen showed a decline within 2 days and no recovery by 4 days. A 40°C treatment allowed no recovery of *P. ramorum* from infected tissue after 2 days. For cold treatments, *P. ramorum* was recovered from infected leaf disks at 0 and –10°C after 7 days. At –20°C, recovery declined rapidly after 1 to 3 days and no recovery was obtained after 4 days. *P. ramorum* showed nearly 100% recovery from leaf disks subjected to a 12-week variable temperature treatment based on ambient summer temperatures in Lewisburg, TN. The results suggest that *P. ramorum* is capable of surviving some highly adverse temperature conditions for at least 7 days both as free chlamydospores in sand and within infected host tissue. Thus, *P. ramorum* present as free chlamydospores or within tissue of infected plants shipped to the eastern United States has the potential to survive some of the adverse conditions encountered in summer and winter in many eastern states.

Phytophthora ramorum Werres, De Cock & Man in ‘t Veld, a recent introduction into the United States, has proven to be a threat to hardwood forests on the west coast, specifically tanoak (*Lithocarpus densiflorus* (Hook & Arn.) Rehder) and *Quercus* spp. (34). This pathogen has a broad host range which is ever-expanding and includes understory plants and commonly grown nursery plants (19,25). Some native eastern plants in the Ericaceae family such as mountain laurel, blueberry, bearberry, and huckleberry are susceptible to infection by *P. ramorum* (43), as well as white oak, chestnut oak, and northern red oak that developed disease following stem inoculations (42). This raises concerns for the health of the hardwood forests in the eastern United States should *P. ramorum* become introduced.

Attempts have been made to determine the risk posed by *P. ramorum* to the eastern

United States using weather data (39,45). However, the utility of efforts to determine the risk of *P. ramorum* spread is restricted by our limited knowledge of the pathogen’s survival ability. Previous work has shown that temperatures supporting mycelial growth of *P. ramorum* in vitro were 2 to 28°C, chlamydospore production 8 to 28°C, and sporangia production 10 to 30°C (12,47). In spite of the wide temperature range for growth shown by *P. ramorum*, harsh winter temperatures encountered in the northern regions of the country may limit its establishment.

Because *P. ramorum* is primarily a foliar pathogen, it is likely to overwinter as hyphae or chlamydospores in stems and leaves on the plant or in abscised leaves found on the soil surface, in leaf litter, or in shallow depths of soil. Therefore, the fungus will be subjected to freezing ambient temperatures, freeze and thaw fluctuations, and desiccation. Similarly, high temperatures and dry conditions encountered in the summers in various regions may limit growth and establishment of *P. ramorum*.

Davidson et al. (8) reported that *P. ramorum* survived the warm, dry summer months in California in attached bay laurel (*Umbellularia californica*) leaves, although levels declined over time. These workers were not able to reisolate *P.*

ramorum from abscised leaves collected from leaf litter. Davidson et al (10) also established a strong link between sporulation on bay laurel and transmission of *P. ramorum* in California forest ecosystems. It was demonstrated (9) that *P. ramorum* chlamydospores could survive a 30-day treatment at 15°C in water or on moist filter paper, but did not survive when initially dried at room temperature and 30% relative humidity for 30 min. Studies by other workers (5,13,29,37,38) have demonstrated that *P. ramorum* chlamydospores are long-lived and can be recovered following placement in potting media after many months. Knowledge of the impact of extreme temperatures on *P. ramorum* survival both as chlamydospores and within host tissue is necessary to predict the ability of the pathogen to overwinter and provide inoculum for continuing epidemics in forest ecosystems. Such knowledge also would help identify constraints to over-summering and overwintering survival, and allow predictions of how widely the pathogen may become established in new regions. This information would be of use to regulators, inspectors, and Forest Service personnel who seek to determine the limits to *P. ramorum* establishment.

We assessed the germinability of *P. ramorum* chlamydospores following exposure to extreme constant temperatures, both high and low, for up to 7 days. We also assessed the recovery of *P. ramorum* from plant host tissue (*Rhododendron* ‘Cunningham’s White’) at extreme constant temperatures for up to 7 days. Leaf disks were incubated in three different soils (sand, sandy loam, and loam) and at two different water holding capacities (WHCs) in each soil type. In addition, we monitored recovery of *P. ramorum* in plant host tissue held under previously mentioned soil and moisture regimes, but subjected to ambient temperatures based on historical weather data for a period of 12 weeks.

MATERIALS AND METHODS

P. ramorum was cultured on 20% V8 media, unless otherwise noted, and maintained at 20°C. Pimaricin-ampicillin-rifampicin-pentachloronitrobenzene-hymexazol (PARPH) (24,31) with 4% clarified V8 juice added was used for chlamydospore germination and leaf tissue isolations. Pr-52 was the isolate used in these studies, and originated from *Rhododendron* spp. in California in 2000. This is a well-characterized isolate belonging to the

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US-1 genotype (22,23) that has been used in many previous studies (12,17,18,20,21, 30,35,41,43).

Chlamydospores. Sand inoculum was produced using the procedure described by Graham (15) and used by Colburn and Shishkoff (6). Mycelial plugs of *P. ramorum* were used to inoculate 250-ml, canted-neck polystyrene tissue culture flasks (BD Biosciences, Bedford, MA) containing 40 ml of 20% clarified V8 juice broth. The flasks were incubated at 20°C in darkness for 14 days, at which time the V8 broth was replaced with 100 ml of sterile distilled water. Cultures then were incubated for two additional weeks, at which time contents were filtered through cheesecloth. The resulting hyphae and spores were added to autoclaved, air-dried then pre-moistened (5% vol/wt) sand (Play Sand; Quikrete Inc., Atlanta) held in Ziploc bags at a ratio of 10 cultures to 1 liter of sand. The sand inoculum was mixed vigorously by hand for 5 min every other day for 7 days and then stored at 20°C in darkness for at least 1 month. Chlamydospore germination levels on PARPH+V8, determined for each batch of inoculum produced, ranged from 40 to 47%. We saw no colonies originating from hyphal fragments. We also could readily observe germ tubes coming directly out of the chlamydospores, assuring that they did not originate from attached hyphal fragments. Chlamydospores were exposed to temperatures of 30, 35, 40, 0, -10, and -20°C in separate experiments. Samples of sand inoculum (5 cm³ each) were placed in 15-ml conical plastic centrifuge tubes and incubated at the above temperatures for 1, 2, 3, 4, and 7 days in replicates of three. A duplicate set of samples was incubated at 20°C as a control. Following incubation, the contents of each tube were transferred to 125-ml polypropylene Erlenmeyer flasks containing 45 ml of sterile 0.2% water agar. The flasks were capped with

plastic caps and shaken by hand to disperse chlamydospores, and 1-ml aliquots were delivered to each of three 9-cm-diameter petri plates of PARPH+V8. Numbers of colonies resulting from germinated chlamydospores were counted under a dissecting microscope over a period of up to 14 days. Experiments were performed three times and data analyzed by analysis of variance using PROC GLM in the SAS statistical package (36).

Leaf disks, constant temperatures.

Leaves were collected from *Rhododendron* 'Cunningham's White' plants maintained in a greenhouse, and surface disinfested for 1 to 2 min in 1% sodium hypochlorite followed by four rinses in distilled water. Ziploc bags containing 1,000 leaf disks (0.32-cm² disks removed using a hole punch) were inoculated by immersion in 300 ml of a sporangia suspension (3,000 sporangia/ml) produced by incubating colony plugs in 1% (sterile) soil extract for 72 h. After 5 days, the sporangia suspension was replaced with distilled water and the 20°C incubation was continued for 4 weeks. The presence of chlamydospores within uncleared leaf tissue was confirmed microscopically prior to use in trials.

Infected leaf disks were incubated in two locally collected soils and in sand (Play Sand; Quikrete Inc.). The sandy loam soil (66% sand, 24% silt, 10% clay; pH 3.7; 6.6% organic matter [OM]) was collected from a hardwood forest near Frederick, MD. The loam soil (37% sand, 39% silt, 24% clay; pH 5.3; 1.4% OM) was collected from a cultivated field at Ft. Detrick, MD used in agricultural research for over 20 years. Soils were air dried and passed through a 2-mm sieve; sand was rinsed and air dried prior to use. Scintillation vials (20 ml) were filled with 10 cm³ of soil or sand. Distilled water was added to adjust moisture levels to 30 and 60% WHC and vial contents were stirred. Equivalent volumetric soil moistures were 0.068 and 0.138 cm³ water g⁻¹ soil., respectively, for sand; 0.394 and 0.750 cm³ water g⁻¹ soil for loam. Three infected leaf disks were added to each vial. Samples were incubated for 1, 2, 3, 4, and 7 days at 30, 35, 40, 0, -10, or -20°C. A 20°C control was included with each of the three runs. Leaf disks were removed from vials following incubation, rinsed, quartered, and plated on PARPH+V8. Plates were examined periodically for up to 14 days. Recovery was characterized as growth of *P. ramorum* from any one of four sections of each leaf disk into the media. Experiments were performed three times.

Data were analyzed by logistic regression modeling the odds of growth (binary response, growth or no growth) on any of the three disks in a vial. The analyses were conducted using the logistic procedure (PROC LOGISTIC) of the SAS statistical package (36) with logit values (natural log

of the odds of growth) as the dependent variable, soil type and WHC as class variables, and temperature and days of incubation as covariates. After the first set of analyses, WHC was dropped from the models (see Results) and the data were reanalyzed with temperature within soil types and days of incubation within soil types as covariates. Single-degree-of-freedom contrasts were written to compare logit values of each soil type at each temperature and days of incubation and to compare slopes between soil types attributable to temperatures and days of incubation.

Leaf disks, variable high temperatures. We examined historical weather records for selected regions of the country where conditions appear to be conducive to infection by *P. ramorum*. Ambient and soil temperature data was acquired from the National Climatic Data Center, Asheville, NC (<http://www.ncdc.noaa.gov>) (32). Lewisburg, TN was chosen because it is representative of regions under high risk of establishment of sudden oak death in the eastern United States (39,45). Temperatures at Lewisburg, TN (Table 1) were found to be intermediate between those for Raleigh, NC and Columbia, SC.

Infected *Rhododendron* 'Cunningham's White' leaf disks in sand, sandy loam, and loam at 30 and 60% WHC were incubated at temperatures representative of average diurnal ambient temperature in Lewisburg, TN from June through August (1982–2001). Temperatures were adjusted on a weekly basis to correspond with historical minimum and maximum means (Table 1). Six plastic containers (2 liters, 19 by 19 cm; Rubbermaid Home Products, Wooster, OH) were filled with 1,500 cm³ of sand, sandy loam, or soil, for a total of 18 containers. Distilled water was added to achieve 30 and 60% WHC. Thirty-six nylon mesh bags containing five infected leaf disks were buried in each container of soil or sand. One container filled with each soil-moisture combination was placed in each of three incubators and arranged randomly. Two incubators were programmed to ramp from the mean minimum to the mean maximum temperature over a 24-h period without lights. The third incubator was maintained at a constant 20°C as a control. Containers were weighed and moisture levels adjusted back to beginning levels two times a week. Seven weeks into the experiment, covers were placed loosely on top of containers to counteract uneven drying due to variable fan speed within incubators.

To sample, three mesh bags containing five leaf disks each were removed from each container once a week. Leaf disks were agitated in distilled water to dislodge soil particles, blotted, cut in four segments, and plated on PARPH-V8 media. Any leaf disks which appeared dry were rehydrated in distilled water for an hour prior to plat-

Table 1. Weekly temperatures used in 12-week variable temperature growth chamber experiments assessing survival of *Phytophthora ramorum* in *Rhododendron* 'Cunningham's White' leaf disks^a

Week	Temperature (°C)	
	Minimum	Maximum
1	16	29
2	17	31
3	17	31
4	18	31
5	19	32
6	18	32
7	19	33
8	19	32
9	18	32
10	17	32
11	17	32
12	17	32

^a Based on average ambient summer temperatures (June to August) found in Lewisburg, TN for the period 1982 to 2001 (32).

ing. Plates were examined periodically for up to 14 days. Recovery was characterized as growth of *P. ramorum* from any one of four sections of each leaf disk into the media. Leaf disks contained an average of 45 chlamydospores internally (based on 40 leaf disks examined), with many more chlamydospores visible on the leaf surface.

RESULTS

Growth of chlamydospores at constant high and low temperatures. For the high-temperature treatments, chlamydospores showed the highest colony numbers at 20 and 30°C, declining recovery at 35°C over the 7-day period, and no recovery at 40°C (Fig. 1A). After an exposure period of 1 day, recovery from the 30 and 35°C treatments was slightly but significantly reduced compared with the 20°C control treatment, while the 40°C treatment differed significantly (via the Waller-Duncan K ratio *t* test, $k = 100$) from the other three treatments, showing nearly zero recovery (Fig. 1A). At days 2 and 4, the 20 and 30°C treatments did not differ significantly from one another, whereas they did at days 3 and 7. High recovery rates were observed at these two temperatures throughout the 7-day period, ranging from 254 to 320 colonies per plate for the 20°C treatment and 217 to 299 colonies per plate for the 30°C treatment. A significant difference was not observed between the 30 and 35°C treatments at day 1, but it was at days 2, 3, 4, and 7, with recovery of chlamydospores at 35°C exhibiting a steady decline over time, from a mean of 281 colonies per plate at day 1 to 0 colonies per plate at day 7. Recovery at 35°C was significantly higher than that at 40°C at days 1, 2, 3, and 4; however, at day 7, both showed no recovery.

For low-temperature treatments, we observed chlamydospore-based colony numbers ranging from 163 to 285 colonies at 20°C and from 142 to 172 colonies at 0°C (Fig. 1B). Little or no recovery was observed at -10 or -20°C (Fig. 1B). The 0°C treatment showed significantly lower chlamydospore growth compared with the 20°C control treatment at days 1, 2, 3, and 7, but not at day 4 (Fig. 1B). The -10 and -20°C treatments never differed significantly from one another throughout, with a range of one to nine colonies observed at -10°C over 1 to 7 days and no colonies for the -20°C treatment at all days.

Recovery from infected leaf disks at constant high and low temperatures.
Leaf disks, high temperatures. *P. ramorum* showed nearly 100% recovery at 20 and 30°C over all 7 days; however, at 35°C, recovery showed a decline within 2 days (Fig. 2). By day 4, there was no recovery from the 35°C treatment in any of the three soils (Fig. 2). A temperature of 40°C was lethal to *P. ramorum* after 2 days and no growth was observed in any treatment at 3, 4, or 7 days. No significant differences in

recovery were observed between 30 and 60% WHC at any time period; therefore, data for the two WHC levels were combined for subsequent analyses. Slope values for temperature within soil types were negative and significant for each soil type, indicating that chances for pathogen regrowth decreased as temperature increased to 40°C. Slope values for days of incubation within soil types were negative and significant for each soil type, indicating that chances for pathogen regrowth decreased as days of incubation increased. Based on logit values, the slope for recovery from loam soil declined significantly more sharply than did the slope for recovery from sandy loam soil. But at 20, 30, and 35°C with incubation for 1 or 2 days, the logit values from loam were significantly greater than logit values from sandy loam. At 20 and 30°C for 3 and 4 days, incubation the logits for loam also were significantly greater than the logits for sandy loam. These results indicate that there were better chances of recovery in loam than sandy loam under these conditions but chances of recovery diminished more quickly with increasing temperature in loam. There were no significant differences between either of the two soil types and sand.

Leaf disks, low temperatures. *P. ramorum* was recovered at high rates from infected *Rhododendron* 'Cunningham's White' leaf disks at temperatures of 20, 0, and -10°C for up to 7 days buried in sand, loam, and sandy loam soils (Fig. 3). However, at -20°C, recovery declined rapidly after 1 to 3 days and no recovery was obtained in any of the treatments tested after 4 or 7 days. As observed for high-temperature treatments, no significant differences were observed between 30 and 60% WHC at any of the time periods tested; therefore, data were combined for subsequent analyses. Slope values for temperature within soil types were positive and significant for each soil type, indicating that chances for pathogen regrowth increased as temperature increased to 20°C. Slope values for days of incubation within soil types were negative and significant for each soil type, indicating that chances for pathogen regrowth decreased as days of incubation increased. At -20°C, incubation in sand for 1, 2, or 3 days gave significantly higher logit values than incubation in loam for the same amount of time, indicating better chances of recovery in sand under these conditions. No other differences between soil types were de-

Fig. 1. Recovery of chlamydospores of *Phytophthora ramorum* under A, high- and B, low-temperature treatments. Error bars represent standard deviation.

tected. No differences in slopes attributable to temperature or days of incubation were detected among soil types.

Recovery from leaf disks at variable temperatures. Recovery of *P. ramorum* from infected *Rhododendron* 'Cunningham's White' leaf disks was at or near 100% for all treatments over the 12 weeks of both experiments. Treatment differences were not observed for soil type or WHC.

DISCUSSION

The results of our experiments show that *P. ramorum* is able to withstand temperatures of 35°C for up to 4 days as free chlamydospores in sand and 40°C for 1 to 2 days within infected *Rhododendron* 'Cunningham's White' leaf disks. The presence of abundant chlamydospores within infected leaf disks was confirmed microscopically, leading us to conclude that chlamydospores were the primary means of survival in leaf disks.

Several previous studies have been performed to investigate survival of *P. ramorum* in soil and infected plant tissue. Linderman and Davis (29) found that *P. ramorum* could be detected in soil held at 20°C in darkness via baiting and direct

plating for up to 6 months following infestation with sporangia, and for up to 12 months following infestation with chlamydospores. Shishkoff (37) found that *P. ramorum* as mycelium or embedded in leaf tissue and buried in soil under greenhouse conditions (approximately 25°C) could be recovered after 654 days. In terms of heat tolerance of *P. ramorum*, Swain et al. (41) reported that treatments of 24 h at 40°C, 2 h at 45°C, and 1 h at 55°C were sufficient to kill 1-cm-diameter *P. ramorum* colonies growing on 5% V8 juice agar.

Our observations of infected rhododendron tissue are similar in that we both report 40°C as a critical temperature for survival, although we found that a longer incubation period (48 h) was required to eliminate recovery from infected rhododendron leaf disks. Swain et al. (41) also studied the effect of composting and heat treatment on infected plant material (artificially and naturally infected *Quercus agrifolia* wood chips, artificially inoculated *Q. agrifolia* stem sections, and artificially infected bay laurel leaves) and found that 2 weeks of oven exposure at 55°C prevented recovery of *P. ramorum* from these substrates. However, lower temperatures and

shorter intervals at a 55°C treatment of plant substrates were not examined in this study, leaving undetermined whether they would have affected recovery.

Harnik et al. (17) reported a 60% recovery rate for *P. ramorum* from artificially infected bay laurel leaves following a 55°C exposure for 1 week, whereas a 2-week treatment at this temperature eliminated recovery of the pathogen. We observed no recovery from infected rhododendron leaf disks exposed to 40°C for 2 days. The difference in our findings could be due to several factors, including differences in inoculation and recovery strategies, incubation methods, the type of plant tissue assayed, and the isolates used in the experiments. In the Harnik et al. study, leaves were infected by wound inoculating bay laurel leaves with an agar slurry of *P. ramorum*. After 10 days of incubation in a sealed container, leaves were subjected to heat treatment. We observed microscopically that substantial numbers of chlamydospores did not form until 2 to 3 weeks after inoculation of rhododendron leaves with sporangia; therefore, we allowed the incubation to continue for 4 weeks. Thus, chlamydospore quantity and age likely

Fig. 2. Results of high-temperature treatments for infected rhododendron leaf disks buried in three different soils at two water holding capacities for 1, 2, 3, 4, and 7 days. Error bars represent standard deviation.

would have varied between our work and that of Harnik et al. (17). Furthermore, they incubated leaves in a moist chamber for 48 h at room temperature to enhance the success of recovery of the pathogen prior to plating on selective medium. Fichner et al. (13) reported that hydration for 3 weeks enhanced recovery of *P. ramorum* from rhododendron leaf disks buried in mesh bags in forest soils. Thus, hydration treatment may activate some chlamydospores to germinate that would not have germinated immediately upon direct plating. In addition, Harnik et al. (17) examined plates for growth of *P. ramorum* from bay leaves over an 8-week period compared with only 2 weeks in our study. We were not able to incubate and read plates for longer than 2 weeks due to growth of *Pythium* spp. from the nonsterile soil used in our experiments. Another difference between the two studies is in the plant material used. Bay laurel leaves are much less succulent compared with rhododendron leaves and may offer the pathogen greater protection from extremes of temperature. Finally, isolate differences might also partially account for differences in results among the various studies. A short-

coming of the current study is that we used only a single isolate of the pathogen (Pr-52), even though the isolate is well characterized (12,17,18,20–23,30,41,43). Swain et al. (41) utilized five isolates (Pr-1, Pr-2, Pr-5, Pr-52, and Pr-102) to assess survival of *P. ramorum* cultures, and a mixture of isolates Pr-52 and Pr-102 to inoculate bay laurel leaves. Harnik et al. (17) used isolates Pr-1, Pr-2, and Pr-5 to inoculate bay laurel leaves that were subjected to a 55°C treatment. Additional isolates should be tested to see if their survival at extreme temperatures varies from what we observed with isolate Pr-52. Future studies also should be performed with additional plant substrates and temperature treatments, including infected bay laurel leaves placed at a range of temperatures, to fully determine the parameters governing high-temperature survival of *P. ramorum* in host tissue.

For other *Phytophthora* spp. subjected to heat treatment, chlamydospores and hyphae of *P. cinnamomi* and a low-temperature isolate of *P. megasperma* were killed in moist soil and infected walnut twigs within 20 min at 45°C (26). However, 30 min were required at 45°C to kill

all propagules of *P. cactorum*. Propagules of a high-temperature isolate of *P. megasperma* were still viable after 30 min at 45°C (26). Coelho et al. (4) reported that temperatures of 47°C for several hours were necessary for populations of *P. nicotianae* chlamydospores to decline to undetectable levels. Enclosure in host tissue may provide chlamydospores some protection from high temperatures and dry conditions. For example, *P. infestans*, normally killed at temperatures >30°C, has been shown to survive in stem tissue exposed to 40°C (27).

Under the variable warm temperatures we tested (Table 1), which represent ambient temperatures in Lewisburg, TN from June to August, we found close to 100% recovery of *P. ramorum* from leaf disks held in sand or soil, indicating that this set of treatments was not sufficient to reduce survival of the pathogen significantly. We also did not observe any difference in recovery levels between 30 and 60% WHC for any of the temperatures or time periods tested. We would expect that incubation in drier soils than tested here would have a detrimental impact on survival of *P. ramorum*. In fact, because *P. ramorum*

Fig. 3. Results of low-temperature treatments for infected rhododendron leaf disks buried in three different soils at two water holding capacities for 1, 2, 3, 4, and 7 days. Error bars represent standard deviation.

appears to be able to tolerate the warm temperatures found in temperate regions, soil moisture may have a larger impact in limiting its establishment in a particular area. More stringent sets of variable temperature parameters need to be tested to determine the effects of fluctuating temperatures and soil moistures as typically occur in nature on survival.

It is not uncommon for various species of *Phytophthora* to overwinter in infected host tissue (1,7,14,16,28,48). We found that *P. ramorum* within infected rhododendron leaf disks showed nearly 100% recovery at 0 and -10°C for up to 7 days in sand, loam, and sandy loam soils. In contrast, incubation of free chlamydospores at -10°C for up to 7 days resulted in little to no recovery. A sharp drop in survival of *P. ramorum* in leaf disks occurred at -20°C , although even at this temperature the pathogen survived in all three soils for up to several days. From these results, we surmise that the threshold for survival of *P. ramorum* in rhododendron tissue under these time periods is between -10 and -20°C , and between 0 and -10°C for free chlamydospores. Ambient mean minimum values in parts of the northeastern United States over a 20-year period were as low as -11°C in Newton, NJ and -18°C in Caribou, ME. Mean soil temperatures measured at a depth of 10.2 cm declined to 0 and -5°C , respectively.

In terms of the ability of other *Phytophthora* spp. to survive cold temperatures, De Bruyn (11) reported that pure cultures of *P. infestans* could survive up to 10 days at -17°C and up to 5 days at -25°C . Dry conditions enhanced survival, and manure was a more favorable substrate than was soil or sand. Benson (1) observed that inactivation of *P. cinnamomi* in cornmeal agar disks occurred by 2, 6, or 16 days of exposure to -6.7 , -3.8 , and -1.4°C , respectively. Chlamydospores in soil were found to be inactivated at about the same rate as mycelium in agar. For *P. lateralis* in OM in soil, no reduction in survival for 20 weeks was noted at 5, 10, or 15°C ; however, at -5°C , a significant decrease in survival occurred within 2 weeks. However, *P. lateralis* was still detected in frozen OM after 16 weeks (33). *P. parasitica* survived in leaf disks exposed to -4.5°C for several days only, and less than 11 days at 0°C (28). *P. parasitica* also failed to survive in leaf disks placed on the soil surface of pots held outdoors, presumably from both cold temperatures and desiccation. Sneh and McIntosh (40) found that sporangia and oospores of *P. cactorum* retained viability in soil held for several hours at -10°C . Thus, our results indicate that *P. ramorum* is very robust in its ability to survive at cold temperatures when compared with other species.

To help predict the temperature limitations that may affect growth and establishment of *P. ramorum*, we identified the

ranges of temperature and exposure periods which prevent recovery of this pathogen, both from within infected host tissue and as free chlamydospores in sand. In comparison with other *Phytophthora* spp., *P. ramorum* shows a strong capacity to withstand both excessively hot and cold temperatures. Based on these results, *P. ramorum* likely should be able to overwinter in many parts of the eastern United States in rhododendron host tissue or as free chlamydospores, and also withstand summer conditions in many parts of the eastern United States. In spite of its apparent ability to survive temperatures characteristic of summer and winter in the eastern United States, there have been no reports yet of *P. ramorum* becoming established in this region in spite of the many nursery finds made in 2006 (44,46). Conversely, in the United Kingdom, *P. ramorum* has been observed to infect oak trees in a natural setting, in close proximity to other infected plant species (2,3). Thus, the potential for *P. ramorum* to move from ornamental hosts to oak in a natural setting has been proven. Thus, a better understanding of the conditions under which *P. ramorum* may survive in host tissue leads to greater understanding of its potential to cause epidemics on valued forest species in diverse ecosystems.

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