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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 Ericaceous 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 oversummering 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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