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Persistence of *Phytophthora ramorum* in Soil Mix and Roots of Nursery Ornamentals

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ABSTRACT
Shishkoff, N. 2007. Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. Plant Dis. 91:1245-1249.

Although most *Phytophthora* species have a soilborne phase that is crucial for infection of roots and for survival away from the host, the details of the soil phase of *Phytophthora ramorum* are not yet fully understood. As mycelium ages, it becomes resistant to sterilization by acidic electrolyzed water (AEW), a product of the electrolysis which can be used as a disinfectant. Colonies of *P. ramorum* could be recovered from moist potting mix or sand for many months, whether buried as infected plant leaf tissue or as mycelium bearing chlamydospores, and the buried material was also resistant to treatment by AEW. There was no significant difference in recovery over time among treatments (sand or potting mix; infected plant tissue or mycelium); after approximately a year, colonies could be recovered at 0.8 to 14.3%. When excised roots were inoculated with *P. ramorum* sporangia and buried in mesh bags in potting mix, the pathogen was recovered from buried roots for at least 8 to 11 months, but it was not clear whether it was surviving as mycelium or chlamydospores. The roots of living plants of *Acer macrophyllum*, *Buxus sempervirens, Camellia oleifera*, *C. sinensis, C. sasanqua*, *Lonicera hispidula, Taxus baccata, Umbellularia californica, Vaccinium macrocarpon, Viburnum davidii, V. tinus, V.* × *pragense, Rhododendron* 'Gloria', and *Syringa vulgaris* were drenched with a sporangial solution of *P. ramorum* and incubated for a month; the pathogen could be recovered from roots of all plants except those of *Buxus sempervirens* and *Lonicera hispidula*. Recovery on selective agar medium (P5ARP) was from both washed and surface-sterilized roots, suggesting that the roots were internally infected. When chlamydospores were placed near roots and observed directly, they were seen to germinate, forming sporangia. Nearby roots became infected, the tips covered with sporangia. Therefore, *P. ramorum* appears to have a soil phase, at least under greenhouse and nursery conditions.

Additional keywords: sudden oak death

Phytophthora ramorum causes stem cankers on oaks and foliar lesions and stem dieback on a number of plants (7). It was first observed in the 1990s in California coastal forests and in Europe on nursery stock (19). Although the distribution of *P. ramorum* in U.S. forests is currently limited to parts of California and Oregon, there is potential for spread with the movement of water, soil, plants, and plant products. By the end of 2004, infected containerized ornamentals were inadvertently shipped from California to at least 40 states (18), leading to the instigation of an Emergency Federal Order, which placed restrictions on the movement of host plants shipped out of California, Oregon, and Washington (2). To make such a quarantine effective, it is essential that the life cycle of the organism be clearly understood.

Zoospores and sporangia infect the aboveground portions of plants, but what happens in the soil is not well understood. In forests, *P. ramorum* has been shown to spread in soil along hiking trails (8), but it isn't clear in what form the pathogen survives and is spread. *P. ramorum* has been shown to survive in buried leaf tissue in forest soils for up to 24 weeks (9). Preliminary data indicated that *P. ramorum* would infect roots under laboratory or greenhouse conditions (6,12,16,17), and it has recently been observed infecting the roots of tanoaks under natural conditions (15).

In addition to sporangia, *P. ramorum* produces chlamydospores, which are structures that are associated with long-term survival in soil in other *Phytophthora* species (10). *Phytophthora cinnamomi* is 2 chlamydospore-forming species that survives in soil, dead root pieces, or living root systems. It also has a wide host range and is a common pathogen in containerized nurseries. When it was introduced to Australian forests in the 1920s, it proved to be one of the most destructive pathogens ever documented (14), and when it was introduced to the southern United States sometime in the late 1800s, it killed off chestnuts in the southern part of their range, chestnut blight removing the rest (1). It persists today as one of the most common *Phytophthora* species encountered in eastern forest soils (3).

The objective of this paper was to examine the soil phase of *P. ramorum* in containerized ornamentals, including the recovery of the pathogen after burial in potting mix and sand under greenhouse conditions and the ability of the organism to colonize living and dead roots of host plants. Better understanding of the pathogen might lead to better detection and eradication.

MATERIALS AND METHODS

Isolate. *P. ramorum* isolate (5-C) used in these experiments was originally recovered from *Camellia sasanqua* 'Bonanza' in California in 2003. It was maintained in sterile water culture or on P5ARP (see below) and inoculated onto and reisolated from *Rhododendron* or *Camellia* every 6 months to maintain pathogenicity. To determine percent recovery during experiments, chlamydospores and plant parts were plated on pimaricin-ampicillin-rifampicin-PCNB agar selective medium (P5ARP) (11).

The culture is maintained at –80°C as part of the international collection of plant pathogens at the National Cancer Institute's Central Repository in Frederick, MD.

Production of chlamydospores. Chlamydospores were produced within leaf tissue or as part of the mycelium produced in sterile water culture. This material was used to study the survival of *P. ramorum* in potting mix and in experiments where they were exposed to acidic electrolyzed water. Chlamydospores in tissue were produced in camellia plants by immersing foliage in a sporangial suspension (approximately 2,000 sporangia/ml water) and then incubating the plants in a dew chamber for 4 days at 20°C. Symptomatic leaves were removed, washed, and placed in a plastic bag lined with a moist paper towel and incubated in the dark at 20°C for 3 weeks, until chlamydospores were clearly visible within leaf tissue when viewed with a zoom dissecting microscope at 20 to 60×. Disks were then cut from tissue using a cork borer (10 mm diameter) for burial in mesh bags. Chlamydospores and myce-

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