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Infectivity and Inoculum Production of *Phytophthora ramorum* on Roots of Eastern United States Oak Species

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Abstract

Widmer, T. L., Shishkoff, N., and Dodge, S. C. 2012. Infectivity and inoculum production of *Phytophthora ramorum* on roots of Eastern United States oak species. Plant Dis. 96:1675-1682.

Little is known about colonization of roots of trees by *Phytophthora ramorum*. We examined zoospore concentration and exposure time needed to infect six *Quercus* (oak) species and the inoculum produced from their roots. Sprouted acorns, exposed to zoospores (3,000/ml) for different times and transplanted to potting soil, were susceptible to infection within 1 h of exposure but root weights were not impacted after 4 weeks ($P = 0.952$). Roots of *Quercus prinus* seedlings, inoculated with sporangia, had 0.6 to 3.2% colonization of the total root mass after 5 months. Neither root lesions nor obvious root sloughing were observed. Inoculum threshold levels were tested by exposing radicles to varying zoospore concentrations for 24 h. Results showed that radicle infection occurred even at 1 zoospore/ml. To test inoculum production, roots were inoculated with sporangia and transplanted into pots. Periodically, samples of runoff were collected and plated on selective medium. Afterward, root segments were plated to calculate percent colonization. After 16 and 35 days, root colonization and inoculum production from oak was lower than that of *Viburnum tinus*, a positive control. This study shows that *P. ramorum* is able to infect sprouted oak acorns and produce secondary inoculum, which may be important epidemiologically.

Phytophthora ramorum Werres, de Cock & Man in't Veld, causal agent of sudden oak death and ramorum blight, is a pathogen of worldwide concern. *P. ramorum* is known to have a broad host range based upon isolations from natural and artificial laboratory infections (57). In the United States, where it is a regulated quarantine organism, *P. ramorum* has a limited distribution in forests in coastal California and Oregon. To date, on the east coast, *P. ramorum* has only been isolated from nursery sites (and, in a few instances, from residential sites where infected nursery stock had been planted) and from adjacent bodies of water (10,24). However, there are many susceptible forest hosts, making the potential for spread to forested areas high. If host range is taken into consideration along with other environmental factors, risk to the Eastern U.S. forests may be higher than for the Western United States (28,29).

Oak species are an important part of many forests around the world, especially in the Eastern United States. The area of the two upland oak groups, oak-hickory and oak-pine, cover 43% of eastern timberland (35). Therefore, it could be devastating to natural forests if *P. ramorum* were to establish in the Eastern United States. Spaulding and Rieske (48) predicted that a nearly 38% decrease in red oak basal area would occur within 10 years of *P. ramorum* invasion in southern Appalachia. A previous study showed that, under artificial conditions, stem and leaf lesions formed on native eastern *Quercus* spp., including *Quercus prinus* L., *Q. alba* L., and *Q. rubra* L., after inoculation with *P. ramorum* (56). However, no previous studies have been conducted to look at the colonization of roots of *Quercus* spp. Root infection is an important infection court of many Phytophthora diseases (17), and may be very important in the life cycle of *P. ramorum*. Indeed, *P. ramorum* has a soil phase that has been demonstrated to infect the root systems of hosts such as tanoak, *Lithocarpus densiflorus* (Hook. & Arn.) Rehd. (38,39,43,45–47,53).

P. ramorum has been considered primarily a pathogen that infects aboveground tissues (leaves, woody shoots, and trunks) of the host (58), with a demonstrated soil phase consisting of infected organic debris, infected roots, and persistent chlamydospores (19,39,43). This ecology of the habitat of *P. ramorum* is not unusual for *Phytophthora* spp. A good comparable example is *P. megakarya*, causal agent of black pod disease of cacao. This species is similarly classified as an aboveground pathogen (13) but has been found on roots in low numbers (37), present in the soil as a reservoir of inoculum (21). For many years, researchers concentrated on aerial plant-to-plant spread; however, it became clear that roots became infected and that rain splash of soilborne inoculum could initiate aboveground infections (3,36). Other examples that have been cited are *P. palmivora* (20) and *P. cactorum* (32). Davidson et al. (15) and Fichtner et al. (18) both demonstrated splash dispersal as a transmission pathway of *P. ramorum* from infested soil to aerial plant parts.

Demonstrating that *P. ramorum* has a soil phase is important epidemiologically because of the potential for movement after rain and in waterways. *P. ramorum* has been found in waterways, such as streams and rivers (14,49), and was recovered at a forested and riparian site 1 km downstream of an infested site that was connected via an ephemeral stream (15). In general, *P. ramorum* has not been considered a serious root pathogen that infects via waterways, in contrast to other species such as *P. lateralis* (26). However, Shishkoff (47) has shown that *P. ramorum* produces inoculum levels from host roots similar to those of other documented root rot *Phytophthora* spp. such as *P. citricola* and *P. citrophthora*. Therefore, it is worth considering that a viable pathway for infection might occur when infested water comes in direct contact with roots.

Sporulation on different hosts is important to study in terms of the host's epidemiological impact on the spread of this pathogen. Various studies have shown that different plant species or cultivars react differently to *P. ramorum* infection in terms of necrosis and sporulation (31,54). However, it is not possible to categorize a plant as conducive to sporulation and, therefore, epidemiologically important based upon the amount of necrotic tissue. Tooley and Browning (55) examined the sporulation of *P. ramorum* on several

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