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Recovery Frequency of *Phytophthora ramorum* and Other *Phytophthora* spp. in the Soil Profile of Ornamental Retail Nurseries

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ABSTRACT

Dart, N. L., Chastagner, G. A, Rugarber, E. F., and Riley, K. L. 2007. Recovery frequency of *Phytophthora ramorum* and other *Phytophthora spp.* in the soil profile of ornamental retail nurseries. Plant Dis. 91:1419-1422.

We tested the hypothesis that inoculum of the aboveground exotic plant pathogen *Phytophthora ramorum* would be limited to the organic layer (top layer of plant debris) of soils at infested retail nurseries located outside of the area where the pathogen has become established in the landscape. To test this hypothesis and compare inoculum levels of *P. ramorum* with levels of other *Phytophthora* spp. in the soil profile, soil cores were collected and sampled from three Washington State retail nurseries at which the soil had previously tested positive for *P. ramorum*. *Phytophthora* was isolated from soil using rhododendron leaves as bait, and pure cultures were obtained and stored on V8 juice agar. Isolates were identified to species using a combination of DNA sequencing of the internal transcribed spacer (ITS) region of rDNA, real-time polymerase chain reaction (PCR) diagnostic testing, and culture morphology. Recovery frequencies were tabulated and compared by species at the organic layer, 0 to 5 cm, 5 to 10 cm, and 10 to 15 cm depth classes. The three most common *Phytophthora spp.* recovered from the soil cores were *P. citricola (32%),* P. *drechsleri* (32%), and *P. ramorum (27%). P. citricola* and P. *drechsleri* were more evenly distributed throughout the soil profile, whereas *P. ramorum* was primarily recovered from the organic and 0 to 5 cm depth class (86% of recoveries). *P. ramorum* was not detected below 10 cm.

Phytophthora ramorum S. Werres & A.W.A.M. de Cock is an exotic plant pathogen responsible for the death of tan oak *(Lithocarpus densiflorus* (Hook. & Am.) Rehd.), coast live oak *(Quercus agrifolia* Nee), and California black oak *(Q. kelloggii* Newb.) in parts of coastal California, tan oak in southwestern Oregon (8,12,13), and ornamental trees such as American southern red oak *(Q. falcate* Michx.) and American northern red oak *(Q. rubra L.)* growing in urban gardens in the United Kingdom and the Netherlands (3,4). This pathogen is also found in horticultural nurseries in North America and Europe infecting a diverse group of hosts from over 16 plant families (14,16,18). *P.* ramorum is generally considered to be an aboveground plant pathogen in nursery systems, where it is known to cause leaf blight and shoot dieback (5,8,10). The pathogen produces sporangia on foliage and twigs that disperse and initiate new infections (8). P. *ramorum* chlamydospores can survive for over a year and sporangia for up to 6 months in potting media (10), and rhododendron plants have been infected when grown in infested potting media in laboratory experiments (9,11). However, the overall significance of the soil phase in the P. *ramorum* disease cycle (or role in the epidemiology of the pathogen) in nursery systems is still unknown.

With continued detections of P. *ramorum* on ornamental nursery stock in parts of North America and Europe (8,14), there has been concern about the potential spread of this exotic pathogen throughout the ornamental nursery trade. On both continents, quarantine efforts are underway to control the spread of *P.* ramorum in nursery stock. In the United States, the Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) has put an Emergency Federal Order into effect requiring nurseries in California, Oregon, and Washington to be inspected and found free of *P.* ramorum before interstate shipping of known and associated host plants (15). Additionally, the Emergency Federal Order requires that when *P.* ramorum is detected in a nursery, a set of procedures known as the Confirmed Nursery Protocol for *P.* ramorum be carried out to eradicate the pathogen (17). After the protocol has been completed, the nursery can be recertified as free of P. *ramorum.* In 2005, changes were made to the USDA-APHIS Confirmed Nursery Protocol requiring soil testing for P. *ramorum* adjacent to containerized nursery plants where the foliage tested positive during nursery inspections (17). Positive soil tests in a number of U.S. retail nurseries have confounded the mitigation process to eradicate the pathogen from these sites.

One of the obstacles in developing mitigation procedures to eradicate this pathogen from nursery soil is the lack of field data on the abundance and depth at which *P. ramorum* can be isolated from soils in nursery settings. Thus far, field research on the survival and recovery of *P. ramorum* from soil depths has focused on situations where the pathogen has established itself in a forest or park-like setting. These studies have demonstrated that *P. ramorum* can be recovered from inoculated leaf disks 6 months after being buried in forest soil in California (7). In a park setting in the Netherlands, the pathogen has been recovered from soil sampled 20 cm deep adjacent to the roots of infested rhododendrons (1).

In retail nursery settings, containerized plants are commonly displayed aboveground, and any inoculum entering the nursery soil presumably comes from fallen, infested leaves or inoculum originating on these plants. Moreover, the roots of containerized plants are generally confined to their pots and are therefore not as likely to provide a potential route *for P. ramorum* to colonize deep into nursery soils as in parks or forests. We hypothesized that in retail nursery settings outside of areas where the pathogen has become established in landscapes, *P. ramorum* inoculum would be limited to the organic layer (top layer of plant debris) of nursery soils. To test this hypothesis, we collected soil cores from three nurseries in Washington State where the pathogen is not known to be established in the natural or urban landscape, and sampled the soil profile for *P ramorum* and other *Phytophthora* spp. A combination of DNA sequencing, real-time polymerase chain reaction (PCR) diagnostic testing, and culture morphology were used to assess the diversity of *Phytophthora* spp. in the soils and to compare the abundance and depth at which P. *ramorum* and other *Phytophthora spp.* were recovered from the soil profile.

MATERIALS AND METHODS

Collecting soil cores. Soil cores (10 x 20 cm) were collected from three Washington State retail nurseries, designated WA-1, WA-2, and WA-3, over localized

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