Recovering Phytophthora ramorum and other Phytophthora spp. from the soil profile of ornamental retail nurseries

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Introduction

With continued detections of P. ramorum on ornamental nursery stock in parts of North America and Europe, there has been concern about the potential spread of this exotic pathogen through the ornamental nursery trade. On both continents, quarantine efforts are underway to control the spread of P. ramorum in nursery settings. 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 shipping known and associated host plants interstate. 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. 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. Positive soil tests in a number of United States retail nurseries have confirmed the mitigation procedures to eradicate the pathogen from these sites.

One of the obstacles in developing mitigation procedures to eradicate this pathogen from nursery soil is that there is no 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. No information has previously been collected on the distribution of P. ramorum in nursery soils. We hypothesized that in retail nursery settings outside of areas where the pathogen has become established in the landscape, 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 3 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 communities in the soils and the abundance and depth at which P. ramorum and other Phytophthora spp. were recovered from the soil profile.

Methods

Collecting soil cores. Soil cores (10x20cm) were collected from 3 Washington state retail nurseries, designated WA-1, WA-2 and WA-3, over localized areas where the soil had tested positive for P. ramorum during Washington State Department of Agriculture (WSDA) nursery inspections conducted in 2005 and/or 2006. Soil cores were extracted from the ground using a foot extraction hole cutter (Par Aide Products Company, Lino Lakes, MN). The cores were then ejected into 2-liter plastic soda bottles of which the tops were cut off and double bagged for transport and storage.

Sampling Phytophthora spp. in the soil cores. Soil was separated along the cores by the top organic layer and then in increments of 0 to 5, 5 to 10, and 10 to 15cm. The organic layer was defined as debris recognizable as plant tissues at the top of the soil profile and was placed in separate sealable plastic bags for each core. The soil cores were then sampled by inverting the 2-liter plastic soda bottle and taking 3 sub-cores (from within the larger 10cm diameter core) using a 2cm diameter soil probe (AMS, American Falls, ID). The 3 sub-cores were then collected at each depth and placed in separate plastic bags designated for the 3 different soil depths for each respective soil core.

Phytophthora spp. was baited from the soil samples in the plastic bags by adding approximately 150ml of sterile tap water and 3 rhododendron leaves (Rhododendron decorum hybrid) (“Caroline”) that had been wounded by slicing the midrib of the leaves. The leaves in the water-soil slurry were incubated 48 hours at 16°C and incubated in germination trays for 10 days at 17-18°C. When a lesion was observed, a section was isolated onto CARP to select for P. ramorum.

Identifying Phytophthora spp. Cultures of P. ramorum were identified based on characteristic mycelium, chlamydospores, aerial mycelia and real-time PCR diagnostic testing using Table 1 conducted by the WSDA Plant pathology Lab (Olympia, WA). Duplicate samples of the other Phytophthora cultures were sent to a commercial laboratory (Laragen Inc., Los Angeles, CA) for DNA extraction, polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region of DNA, and complete sequencing of the ITS using ITS 4 and ITS 5 primers. The sequences were then compared with published sequences. Identifications based on ITS sequences were confirmed by verifying that the colony characteristics and distinctive microscopic morphology and reproductive structures were consistent with published literature.

Data analysis and statistics. A record of each Phytophthora culture recovered was entered into a spreadsheet and sorted by nursery, core, depth class, and species. Chi-square analyses for independence were conducted with P. ramorum recovery frequencies and those of other Phytophthora spp. at the 4 soil depth classes to assess P. ramorum was distributed among the soil depth classes differently than other species.

Results

A total of 69 soil cores were sampled from the 3 nursery sites (32 from WA-1, 20 from WA-2 and 17 from WA-3). The soil types at WA-1, WA-2 and WA-3 were loam, gravelly sandy loam and gravelly sand, respectively. The organic layer of the soil cores for all three nurseries ranged from 1 to 5cm. At all three nurseries the soil was moist at the time of sampling and the cores were sampled from areas with medium to poor drainage. In all, 187 Phytophthora isolates were recovered that had a unique soil core, depth class, and species combination. Forty-one percent of these were from the organic layer, 28% from the 0 to 5cm depth class, 16% from the 5 to 10cm depth class, and 15% from the 10 to 15cm depth class. Forty-two percent of the Phytophthora isolates were recovered from WA-1, 34% from WA-2, and 26% from WA-3. On average, a higher percentage of the total Phytophthora isolates recovered from each nursery came from the upper depth classes (Fig. 1). The 3 most common Phytophthora spp. recovered from the soil cores were P. citricola (32%), P. drechsleri (12%) and P. ramorum (27%). Other Phytophthora spp. recovered included P. gonapodyides and P. cactorum. Over 100 Pythium isolates were recovered from the soil cores as well. Using BLAST search on GenBank, ITS sequences from a subset of these isolates most closely matched ITS sequences of isolates previously identified as Pythium spinellum, P. delicius, P. anulatum, and P. cinctum. P. ramorum was recovered at all three nurseries a total of 28 times from unique soil core and depth class combinations (Fig. 2). A majority (86%) of these isolates were recovered from the organic and 0 to 5cm depth class and P. ramorum was detected as deep as the 5 to 10cm depth class (14% of recoveries). In contrast to P. ramorum, P. citricola and P. drechsleri were recovered more consistently throughout the soil profile (Fig. 3). A chi-square test for independence was conducted with P. ramorum and P. drechsleri, comparing recovery frequencies at the 4 soil depth classes. The chi-square test with P. ramorum and P. cinctica was significant at P=0.01 (df=3, d.f=3) and the chi-square test with P. ramorum and P. drechsleri was significant at P=0.005 (df=3, d.f=3).

Discussion

Overall, more Phytophthora recoveries were observed from samples taken at the upper levels of the soil profile (Fig. 4). When broken down to the species level, differences (chi-square tests significant to a value of P ≤ 0.01) were observed in the recovery frequency of P. ramorum with those of P. citricola and P. drechsleri at different soil depth classes (Table 2). A record of 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 5cm depth class (Fig. 3). These differing trends may be explained by differences in how long these species have been at the nursery sites. Compared to the recent introduction of P. ramorum, P. citricola was described in the United States as early as 1932 and has been reported on a diversity of hosts sold as ornamental nursery stock including Rhododendron spp., Japanese andromeda (Pieris japonica), lilac (Syringa vulgaris), California live oak, white fir (Abies concolor) and Douglas-fir (Pseudotsuga menziesii). Likewise, P. drechsleri was reported in the United States as early as 1929 and has been reported on common ornamental hosts such as heather (Calluna vulgaris), Azalea (Azalea indica) and Douglas-fir. P. ramorum was first reported in North American nursery stock in 2005 which may explain why the inoculum levels have not built up at deeper levels in the soil profile. Additionally, P. ramorum is known as an aboveground pathogen and therefore its inoculum may not be as well adapted to perseverance and surviving deep within the soil profile as P. citricola and P. drechsleri which are primarily root pathogens in a network of hosts. Since P. ramorum was recovered as deep as the 5 to 10cm depth class (Fig. 2), we reject the hypothesis that this pathogen is limited to the organic layer of nursery soil. It is likely that spores from the foliage of nursery stock have percolated into the soil matrix during precipitation events or that chlamydospores embedded within decomposing organic debris on the soil surface have been washed into the soil profile by foot traffic or irrigation. The recovery of P. ramorum as deep as the 5 to 10cm depth class indicates that soil treatments at nurseries where the pathogen is detected in soil should aim to remove or kill inoculum to a depth of at least 10cm. To minimize the potential for P. ramorum soil positives at retail nurseries, nursery personnel should consider storing and displaying soil samples from nurseries to become established at lower levels of the soil profile.

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