

# High Recovery Rate of *Phytophthora* from Containerized Nursery Stock Pots at a Retail Nursery Highlights Potential For Spreading Exotic Oomycetes

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In 2005, an addition was made to the USDA-APHIS Confirmed Nursery Protocol (4) for the quarantined plant pathogen, *Phytophthora ramorum* (5), requiring soil testing adjacent to containerized nursery plants testing positive during nursery inspections. Since this change, *P. ramorum* has been detected in nursery soils under symptomatic and asymptomatic known hosts as well as asymptomatic non-host containerized plants on a number of occasions. This has raised several questions: (i) are containerized non-host asymptomatic plants likely to transport inoculum of *P. ramorum* if sold to the public; (ii) if these plants are carriers of *P. ramorum*, where is the pathogen present; and (iii) what is the occurrence and distribution of other *Phytophthora* species on containerized nursery stock?

In an effort to elucidate these questions, we collected and sampled for *Phytophthora* species from asymptomatic host and non-host containerized nursery stock from a nursery in western Washington state where the soil had tested positive for *P. ramorum* during Washington State Department of Agriculture (WSDA) nursery surveys conducted during the fall of 2005.

## Description of the Site and Containerized Plant Collection

On August 1st, 2006, 57 containerized asymptomatic known host and non-host plants of *P. ramorum* were double-bagged and transported to a laboratory for processing. Containerized plants were collected from areas where soil had tested positive for *P. ramorum* during WSDA nursery inspections. Containers (plastic pots) ranging in size from 15 to 30 cm in diameter had been displayed directly on loamy soil with a 1 to 5 cm organic layer (Fig. 1). Known host species included one dogwood (*Cornus* sp.) and one *Pieris japonica*. All the other plants are currently classified as non-hosts and included sedges (*Carex siderostica*), ferns (*Dryopteris* spp.), *Rubus* sp., and crape myrtle (*Lagerstroemia indica Tuscana*). Soil directly beneath each pot was also collected and placed into plastic zip-lock bags.



Fig. 1. Containerized plants displayed directly on loamy soil in a nursery.

## Recovery and Identification of *Phytophthora* spp. from Containerized Pots and Soil Beneath the Plants

In addition to the soil that was collected beneath each pot, soil and organic debris that were caked onto the side and bottom of each containerized pot were collected by scraping and swabbing it into plastic zip-lock bags. Soil was also collected and stored the same way from the upper, middle, and lower regions of the root ball inside each pot.

*Phytophthora* was baited from the soil samples in the zip-lock bags by adding 150 ml of sterile tap water and 3 rhododendron leaves (*Rhododendron decorum* hybrid 'Caroline') and incubating for 48 hours at 16°C and then removing the rhododendron leaves from the water and incubating in a single layer at 17 to 18°C. The leaves were checked periodically for developing lesions. When a lesion was observed, a section was isolated on corn meal agar amended with ampicillin, rifampicin, and pimarinic acid (CARP) to select for *Phytophthora*. Pure cultures were then made by sub-culturing to V8 juice agar and storing at 16°C.

Cultures of *P. ramorum* were identified based on characteristic mycelia, chlamydospores and real-time PCR diagnostic testing using Taqman. Other *Phytophthora* spp. were identified by amplifying and sequencing the ITS region of rDNA and comparing with published sequences.

### Distribution of *P. ramorum* and Other *Phytophthora* spp. on Containerized Nursery Stock

*Phytophthora* was recovered from the soil collected below 90% of the containerized plant pots, the side of 10% of the pots, the bottom of 5% of the pots, and 8% of the root balls. The most frequently recovered species included *P. citricola*, *P. drechsleri*, *P. gonapodyides*, and *P. ramorum*.

*P. ramorum* was recovered from the soil collected directly below 6 (12%) of the containerized plants. These included 1 dogwood, 1 wood fern, and 4 crape myrtles. Additionally *P. ramorum* was repeatedly recovered from the soil associated with the lower and middle regions of the root ball of a single crape myrtle. No *P. ramorum* was recovered from rinsed or surface sterilized crape myrtle roots. The recovery of *P. ramorum* from the root ball of the asymptomatic non-host crape myrtle was unexpected. This plant was from an out-of-state production nursery and it is estimated the containerized plant had been on site for 5 to 10 years. It is not clear whether the pathogen was introduced into the crape myrtle pot after it was brought onsite or if chlamydospores in infested potting mix were able to survive over the past several years. Research has demonstrated the ability of *P. ramorum* chlamydospores to survive for over a year and sporangia for up to 6 months in potting media under experimental conditions (3). There are also published reports of *P. ramorum* being isolated from asymptomatic roots of *Rhododendron* spp. (1,2). Although we failed in our initial attempts to isolate *P. ramorum* from the roots of the crape myrtle plant, we are currently attempting to recover *P. ramorum* by baiting from rinsed and surface sterilized roots and conducting root inoculation experiments of healthy plants to determine if *P. ramorum* is capable of causing asymptomatic infection of crape myrtle roots.

The recovery of *P. ramorum* from the potting media of an asymptomatic non-host underscores the need for continued research in nursery systems to understand the epidemiology of this pathogen. The recovery of several *Phytophthora* spp. from the side and bottom of pots clearly demonstrates a potential for exotic and endemic oomycetes to be transported on asymptomatic nursery stock and highlights the importance that best management practices are followed by retail nurseries (e.g., display plants only on well drained gravel, cement, asphalt or weed mat). From a regulatory prospective this study suggests that containerized plants in direct contact with soil infested with regulated *Phytophthora* spp. have the potential to move inoculum. Under these conditions even non-host plants should be considered when developing mitigation measures for nurseries in which *P. ramorum* is detected in soil.

### Literature Cited

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