From Forest Nursery Notes, Winter 2008

© 118. Root and stem infection of *Rhododendron* from potting medium infested with *Phytophthora ramorum*. Parke, J. L. and Lewis, C. Plant Disease 91:1265-1270. 2007.

Root and Stem Infection of Rhododendron from Potting Medium Infested with *Phytophthora ramorum*

J. L. Parke, Department of Crop and Soil Science and Department of Botany and Plant Pathology, and **C. Lewis,** Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331

ABSTRACT

Parke, J. L., and Lewis, C. 2007. Root and stem infection of rhododendron from potting medium infested with *Phytophthora ramorum* . Plant Dis. 91:1265-1270.

Phytophthora ramorum has been detected in soil and potting media, but the potential for root infections is not fully understood. To determine whether the root system could become infected and transmit disease, rhododendron 'Nova Zembla' plants grown from rooted cuttings and native Pacific rhododendron (*Rhododendron macrophyllum*) plants grown from seed were transplanted into a potting medium artificially infested with *P. ramorum*. Inoculum consisted of V8-broth-vermiculite cultures of *P. ramorum*, chopped infected leaves, or zoospores. Plants were watered from the bottom to prevent splash dispersal of inoculum onto stems and foliage. Both infested amendments and applications of zoospores resulted in plant mortality within 3 to 7 weeks. *P. ramorum* was isolated from hair roots, large roots, and stems above and below the potting medium surface. Noninoculated control plants remained healthy and did not yield *P. ramorum*. Epifluorescence microscopy of tissue culture plantlets inoculated in vitro revealed attraction of zoospores to wounds and root primordia, and colonization of the cortex and vascular tissues of roots and stems, including the xylem. Transmission of *P. ramorum* from infested potting media to stems via infected, symptomless root tissue demonstrates the need to monitor potting media for presence of the pathogen to prevent spread of *P. ramorum* on nursery stock.

Additional keywords: dose-response, ramorum shoot dieback, sudden oak death

Phytophthora ramorum causes sudden oak death on certain members of the Fagaceae (22), and ramorum shoot dieback and ramorum foliar blight on more than 100 plant taxa, including numerous woody ornamental species (8,28). Originally described as a pathogen of rhododendron and viburnum in Germany and the Netherlands (30), the pathogen is widespread in European nurseries and has been detected in more than 100 private gardens or public greens in the United Kingdom, The Netherlands, and Norway. In 2004, more than 2 million plants potentially infested with *P. ramorum* were shipped from a few large West Coast nurseries in the United States. *P. ramorum* subsequently was detected in 171 nurseries and retail garden centers in 20 states. In 2005, 99 nursery-related sites in seven states were positive for *P. ramorum*. To date in 2006, *P. ramorum* was detected in 56 sites in 11 states: 1 in AL, 26 in California, 1 in Connecticut, 2 in Florida, 1 in Georgia, 1 in Indiana, 1 in Maine, 1 in Mississippi, 13 in Oregon, 1 in Pennsylvania, and 8 in Washington (28). Many of these sites represent recurrent, or incompletely eradicated, infestations.

In an effort to restrict the movement of infected plant material and prevent the spread of the pathogen, the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) imposed an Emergency Federal Order that requires California, Oregon, and Washington nurseries shipping host and associated host nursery stock interstate to be inspected, sampled, and certified as free from *P. ramorum*. Protocols for the sampling and testing of nurseries require that at least 40 samples from symptomatic plants (or asymptomatic plants, if no plants have symptoms) be tested for *P. ramorum* using a genus-specific enzyme-linked immunosorbent assay prescreen, followed by nested polymerase chain reaction (PCR) analysis or culture isolation. Samples to be tested consist of only leaves and stems but not roots or potting media.

P. ramorum is considered to be a pathogen of aerial plant parts (22); however, it has been recovered from soil and streams in infested forests (9,26,27), recirculating irrigation systems in infested nurseries in Germany (25), container media (11), and field soil and water from a retention pond at a retail nursery (29). It also has been recovered from asymptomatic roots of nursery-grown rhododendrons exhibiting stem lesions and foliar symptoms (3) and from roots of container plants inoculated with chlamydospores (6,7,23). It is not known whether infested leaves incorporated into soil or infested potting media could serve as a source of inoculum for subsequent plant infections; however, if so, these could provide pathways for pathogen spread that are not being tested currently in the nursery stock certification process. The objective of this study was to determine whether plants grown in potting media infested with *P. ramorum* could become infected through the root system, leading to pathogen spread above the potting medium surface. A secondary goal was to determine which tissues become colonized by *P. ramorum*. Preliminary reports of this work have been published (15,20).

MATERIALS AND METHODS

Isolates. Two *P. ramorum* isolates were used in these studies. Isolate 03-74-N11-A (A1 mating type, North American genotype) was obtained from Dr. Nancy Osterbauer of the Oregon Department of Agriculture. It was isolated from an infected rhododendron cv. Unique from an infested nursery in Clackamas County, OR in 2003. Isolate 4143 (A2 mating type, North American genotype) originated from a native *Rhododendron macrophyllum* plant in Curry County, OR in 2001. Reference cultures have been deposited in the American Type Culture Collection.

Inoculum. Three types of inoculum were tested. V8 broth-vermiculite cultures and chopped, artificially infested rhododendron leaves were applied as amendments to potting media incorporated at the time of transplanting. In separate experiments, zoospore suspensions were applied to the surface of the potting media, or used for dipping roots of tissue culture plantlets. These inocula are described more fully below.

Water in all inoculum preparations was reverse osmosis purified water (Barnstead/Thermolyne, Dubuque, IA). V8 broth-vermiculite inoculum was produced by mixing 1,500 ml of dry, medium-sized horticultural vermiculite with 750 ml of clarified V8 juice broth (24) in 2-liter Erlenmeyer flasks, and autoclaving twice at 24-h intervals. Fifteen 6-mm-diameter plugs from the margins of 2-week-old *P. ramorum* cultures were added to the flasks and incubated at room temperature for 1

Corresponding author: J. L. Parke
E-mail: Jennifer.Parke@oregonstate.edu

*The *e*-Xtra logo stands for "electronic extra" and indicates that Figure 1 appears in color in the online edition.

Accepted for publication 28 April 2007.

doi:10.1 094/PDIS-91 -10-1265