

Pathways of Infection for *Phytophthora ramorum* in *Rhododendron*¹

Carrie D. Lewis² and Jennifer L. Parke²

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Abstract

The lack of knowledge regarding infection biology of *Phytophthora ramorum* limits our understanding of its ecology and epidemiology. Pathways of infection in *Rhododendron* ‘Nova Zembla’ were investigated using tissue culture plantlets and 3-year-old container plants inoculated with zoospore suspensions (6×10^4 zoospores mL⁻¹) or mycelial plugs of Oregon nursery isolate 03-74-N11A. The histology of inoculated roots, stems and leaf surfaces was evaluated to identify pathways by which *P. ramorum* infects and colonizes plants. To observe infection, inoculated roots, stems, and leaves were examined by means of epi-fluorescence, scanning electron and scanning laser confocal microscopy.

To follow infections initiated from roots into above-ground plant parts, container plants were repotted in a 2:1 mixture of potting soil and V8-vermiculite inoculum of *P. ramorum*. Leaves never touched the potting media, and plants were watered from the bottom only. Leaves wilted and collapsed within 3-6 weeks and upwardly-expanding stem lesions became apparent within 4-6 weeks. *P. ramorum* was recovered through both isolation onto PAR selective medium, and multiplex PCR analysis from symptomatic roots, stems and petioles. Control plants not inoculated with *P. ramorum* remained healthy and did not yield *P. ramorum*. Samples from stems, petioles and below-ground tissues were used for microscopy and PCR analysis. PCR analysis indicated *P. ramorum* was present in roots, stems, and petioles. Tissue samples for microscopy were fixed, embedded, sectioned, stained with 0.01 percent Calcofluor White MR2 and viewed with epi-fluorescence. Preliminary observations of root tissues indicated the presence of *P. ramorum* hyphae on the root surface. Microscopy of stem tissues indicated the presence of *P. ramorum* hyphae in primary xylem at the leading edge of infection. More advanced stem infections indicated the presence of hyphae in pith cells, primary and secondary xylem (*fig. 1*), and chlamydospores in the cortex.

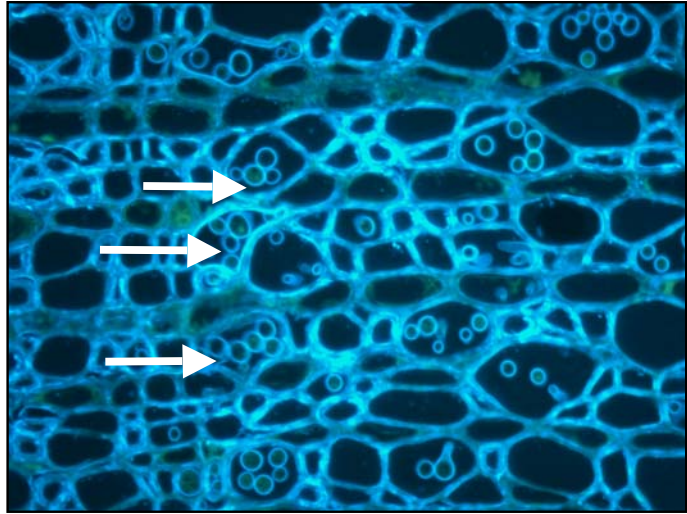
To evaluate root infections, rooted tissue culture plantlets were inoculated by submersing 5-cm sections of rooted stems in a zoospore suspension for 4 or 48 hr. Tissues were fixed, embedded, sectioned, stained with 0.01 percent Calcofluor White MR2 and viewed with epi-fluorescence. Hyphae from germinating cysts were observed penetrating root primordia both inter- and intra-cellularly. Zoospores

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² Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331 USA; (541) 737-4347; lewiscar@onid.orst

congregated near root primordia, emerging lateral roots, and wound sites. After encystment, germination appeared to be directed toward those sites.

Figure 1- Secondary xylem cells from above-ground stem tissue of rhododendron grown in potting media artificially infested with *P. ramorum*. Cross-sections of hyphae (white arrows) can be seen inside individual cells one month after inoculation.



To observe infections inside root tissues, tissue culture plantlets were inoculated by submersing the rooted portion of stem in a zoospore suspension for 1 hour and then incubating for one week in moist paper towels. Roots were removed, stained with 0.01 percent Calcofluor White MR2 and viewed by scanning laser confocal microscopy. Hyphae from germinating cysts oriented towards emerging lateral roots, penetrated the root epidermis, formed hyphae inside root tissue and produced sporangia.

To observe and follow spread of infection in container plants, leaves were wounded and inoculated with mycelial plugs. Plants were grouped by two treatment methods: leaves were inoculated either on the leaf midrib, or on the leaf blade halfway between the midrib and the margin. Two leaves from each plant were inoculated: one young leaf from new growth and one mature leaf from older growth. After 1 to 2 weeks, inoculation near the midrib resulted in more rapid development of leaf necrosis as compared to inoculation of the leaf blade. Infections initiated in young leaves spread through petioles and into stems of new growth, and the necrosis appeared to spread equally up and down the stem as well as into petioles of adjacent leaves. Removal of the leaf directly above the stem lesion revealed discolored vascular bundles in the leaf scar. Infections initiated in mature leaves appeared to stay within the leaf without advancing into petioles. Lesions or necrosis were never observed on non-inoculated but wounded control plants. Stems from new growth and all leaves were plated onto PAR selective medium. *P. ramorum* was isolated slightly beyond the lesion margins. Isolations from older stems indicated that *P. ramorum* spread into stem tissue from leaf inoculation sites even when petioles were not necrotic.

A time-course study was conducted to observe the development of infection on abaxial leaf surfaces. Leaves from tissue culture plantlets were inoculated with a zoospore suspension and incubated for 1.0, 1.5, 2.0 and 2.5 hrs. Leaves were fixed and critical point dried in preparation for scanning electron microscopy. SEM images of leaf surfaces showed that germinating cysts were occasionally found associated with stomata, but stomata are not required for infection. Germinating cysts were more

abundant at wound sites than stomatal openings. Some cyst germ tubes had structures at the growing tip that resembled appresoria, but their function is unclear.