

Survey of Eastern U.S. Native *Rhododendron* spp. for Antagonistic Endophytes Towards *Phytophthora ramorum*¹

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Abstract

Rhododendron maximum L. and *R. catawbiense* Michx. are two species that are native to the eastern United States. They can be found throughout the Appalachian Mountain range and during bloom are very important tourist attractions. *Phytophthora ramorum* is known to be pathogenic to both species, although no symptoms have been observed in wild habitats in the eastern United States. Endophytic fungi are known to have a symbiotic relationship with their host, including protection against pathogens. It was the purpose of this study to survey natural stands of *R. maximum* and *R. catawbiense* in the eastern United States in order to understand what endophytic fungi are present and whether they have the potential to protect against *P. ramorum* infection.

In 2009, leaves of *R. maximum* and *R. catawbiense* were collected in four distinct locations in Virginia, West Virginia, and Pennsylvania. At each location, three mature leaves that did not show any visible signs of necrosis were collected from 10 different plants. The leaves were stored in a cooler and taken back to the laboratory to be processed within 1 week. Leaves were surface sterilized for 1 minute in 70 percent ethanol and rinsed three times in sterile water for 10 minutes each time. After drying, five 11 mm-diameter disks were aseptically cut from each leaf with a cork borer and plated on water agar supplemented with streptomycin (50 mg/L). The plates were stored at 20 °C. Over time, the plates were observed and fungal mycelium growing from the disks was individually transferred to half-strength potato dextrose agar (1/2PDA). Each collected isolate was screened for antagonistic activity towards *P. ramorum* by a dual culture assay. Individual plugs of the fungal isolate were transferred to a 1/2PDA plate containing a plug of *P. ramorum* isolate WSDA-1772 (NA1 mating type), which were stored at 20 °C. After 1 week, the plates were observed for any obvious growth inhibition of the *P. ramorum* colony. Three plates were prepared for each fungal isolate. The fungal isolates that showed some antagonistic activity were tested further in the same dual culture assay described above. Five plates were prepared for each repetition and there were three repetitions per fungal isolate. The antagonistic activity was quantified by calculating the antagonistic index (AI). The AI was determined by subtracting the length of the ray of the *P. ramorum* colony growing towards the fungal isolate from the average length of the three rays of the *P. ramorum* colony growing in the other directions (RM) and dividing the result by RM. Therefore, the closer the value is towards one, the higher the AI.

A total of 631 fungal endophyte cultures were originally isolated from the leaves of the two *Rhododendron* spp. at the four locations surveyed. These isolates were grouped into 72 different types based solely on colony characteristics. Preliminary screening identified 118 cultures that demonstrated some antagonistic activity towards *P. ramorum*, which were tested in detail to determine their AI. All isolates, except one, had a statistically higher ($P < 0.05$) AI value than the control. Values for AI ranged from 0.502 to 0.04. Location, group type, and the *Rhododendron* species, were all significant factors in the AI. These results show that endophytic fungi found in leaves of wild *Rhododendron* spp. have the potential to inhibit the growth of *P. ramorum*. Further work will continue to identify these species and determine what role they may play in protecting the plant from *P. ramorum* infection.

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