First Report of *Phytophthora cambivora* on Hybrid Rhododendron in North Carolina

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A survey of 13 nurseries growing *Rhododendron* spp. was conducted in May 2003 to detect the presence of *Phytophthora ramorum* in North Carolina nurseries as part of the 2003 Sudden Oak Death Pilot National Survey. *P. ramorum* was not detected in any of the nurseries by either isolation from symptomatic plant pieces on PARP selective medium (5) or by a nested-PCR assay (7). However, *P. cactorum*, *P. citricola*, and *Phytophthora* spp. were recovered. Identification of *P. cactorum* and *P. citricola* was based on both morphological characteristics (10) and a RFLP-identification method that utilizes a set of primers, ITS5 and ITS4, to amplify ribosomal DNA and the internal transcribed spacer (ITS) region (9). Both *P. cactorum* and *P. citricola* are known blight and dieback pathogens of rhododendron in the USA and elsewhere (1,3).

A third group of 39 isolates from rhododendron at five of the thirteen nurseries surveyed accounted for about 40% of all of the isolates of *Phytophthora* spp. recovered. Isolates of *Phytophthora* spp. in the third group all produced non-papillate sporangia with external and internal proliferation after several weeks in culture on cornmeal agar, as well as in a 2% soil extract solution. No oospores were observed in single-isolate cultures of four representative isolates, originally recovered from hybrid rhododendron cvs. Catawbiense Boursault and English Roseum, when grown on cornmeal, V8, or hemp seed agars. Oospores were observed after 10 days on V8 agar when the four isolates were paired with A2 isolates of *P. nicotianae*. In subsequent pairings with A2 isolates of *P. cambivora* (done by the first author in the lab of S. N. Jeffers, Clemson University) on amended and clarified V8 agar (4) at 25°C in the dark, typical oogonia with bullate protuberances, amphigynous antheridia and plerotic oospores that averaged 40 µm (s.d. 4.01 µm) were observed (Fig. 1). Consequently, all four isolates were A1 compatibility type and had morphological characters consistent with those of *P. cambivora* (10).

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**Fig. 1.** Bullate oogonium of *Phytophthora cambivora*. Note the elongated oogonium stalk with subtending bicellular amphigynous antheridium and the plerotic oospore. Bar is 40 micrometers.
The RFLP pattern of five representative isolates of *Phytophthora* spp. was uniform, but it did not match that of any of the species of *Phytophthora* described by Ristaino et al. (9). Therefore, a second PCR-based identification method (PhytID, www.phytid.org) utilizing primers ITS6 and ITS4 and the restriction enzymes *Alu*I and *Msp*I was used with the final concentration of MgCl$_2$ in the master mix adjusted to 2 mM (2). With resulting fragment bands of $\geq 50$, 180, 750 and 115, 145, 165, 220, 295, 410 base pairs for *Alu*I and *Msp*I, respectively, a 100% match with *P. cambivora* was found in the PhytID database confirming the identity of our isolates of *Phytophthora* spp.

Koch’s postulates were completed for the four representative isolates of *P. cambivora* by inoculating at least three sets of intact leaves and stems per isolate on plants of hybrid rhododendron cv. Roseum Elegans with 7-mm diameter cornmeal agar disks containing mycelium of each isolate. Agar disks were held in place on the leaves and stems with Parafilm M (Fig. 2). The plants were placed in a greenhouse under intermittent mist that kept a film of water on the foliage. Inoculation of expanding leaves and stems resulted in necrotic tissue within 3 days (Fig. 3). Within 13 days, necrosis had progressed several centimeters down the stems of the expanding shoots on plants inoculated with three of the four isolates (Fig. 4). Ambient greenhouse temperatures ranged from 11 to 33°C and averaged 26.8°C over the 13-day period. *P. cambivora* was re-isolated from the necrotic tissue by culturing on PARP medium. Agar disks of each isolate also were placed on the adaxial or abaxial side of both 10-mm-diameter, mature and immature disks cut from Roseum Elegans leaves and incubated in moist chambers on a laboratory bench. By 12 days post inoculation, all four isolates caused necrosis. One isolate was particularly aggressive infecting both adaxial and abaxial sides of both mature and immature leaves. The other three isolates infected adaxial and abaxial sides of immature leaf disks but only the adaxial side of mature leaf disks.
This is the first report of *P. cambivora* on *Rhododendron* spp. in North Carolina. The fungus, however, was isolated by Osterbauer et al. (8) in the same year as our survey, from rhododendron growing in Oregon nurseries. In 1937, White (11) associated *P. cambivora* (considered by White as synonymous with *P. cinnamomi*) with root rot and wilt of rhododendron in New Jersey nurseries, whereas we found it causing a leaf blight and stem dieback. The blight symptoms we observed were similar to those reported for *P. cambivora* on hybrid rhododendron in Denmark (6). Because the Sudden Oak Death Pilot National Survey was focused on foliage symptoms caused by *P. ramorum*, we did not collect root or medium samples from plants with blight and dieback symptoms to determine if *P. cambivora* also may have been causing root or crown rot on rhododendron.

**Literature Cited**