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10-2005

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Repository Citation

Testa, Antonino; Schilb, Mikael; Lehman, Jeffery S.; Cristinzio, Gennaro; and Bonello, Pierluigi, "First Report of Phytophthora Insolita and P. Inflata on Rhododendron in Ohio" (2005). *Biology and Earth Science Faculty Scholarship*. 7.

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plant disease

Editor-in-Chief: Mark L. Gleason

Published by The American Phytopathological Society

October 2005, Volume 89, Number 10
Page 1128
<http://dx.doi.org/10.1094/PD-89-1128B>

Disease Notes

First Report of *Phytophthora insolita* and *P. inflata* on *Rhododendron* in Ohio

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During August 2003, we conducted a statewide survey of rhododendrons to determine if *Phytophthora ramorum* was present in Ohio ornamental nurseries. In total, 240 samples were randomly collected in 12 nurseries throughout Ohio from rhododendrons showing foliar necrotic lesions and twig dieback symptoms. The samples yielded 51 *Phytophthora* spp. isolates on PARP-V8 agar. The internal transcribed spacer (ITS) region of all isolates was amplified using the universal primers ITS1 and ITS4 and was sequenced. Consensus sequences from sense and antisense were then blasted against the GenBank database, allowing for the identification to species of ~80% of all isolates. These identifications, and the ~20% unknowns, were confirmed using blind morphological tests on the basis of the following parameters: colony morphology; shape and dimensions of sporangia and type of papillae; dimensions of oogonia and oospores; type and position of antheridia; presence or absence of chlamydospores; presence or absence and morphology of hyphal swellings; and growth rate at 35°C according to the Revisited Tabular Key of the species of *Phytophthora*(1). No *P. ramorum* was detected among the isolates; however, *P. cactorum*, *P. citricola*, *P. citrophthora*, and *P. nicotianae* were detected. We also found two occurrences of *P. inflata* Caros & Tucker and one of *P. insolita* Ann & Ko. (*P. inflata*: e -value $\leq e^{-179}$, identities $\geq 95\%$; *P. insolita*: e -value = 0.0; identities = 95%.) *P. inflata* was isolated from two tissue types, a dead twig and a necrotic leaf tip. *P. insolita* was isolated from a

necrotic leaf tip. Identity of the two species was confirmed morphologically using the parameters listed above as well as the following measurements ($N = 40$; all in μm) (1): *P. inflata* - sporangia: 40×24 ($[24 \text{ to } 68] \times [18 \text{ to } 34]$); oogonia: 34.6 (28 to 40); oospores: 30.8 (25 to 38); *P. insolita* - sporangia: 42×28 ($[34 \text{ to } 56] \times [22 \text{ to } 38]$); oogonia: 32 (26 to 36); oospores: 26 (22 to 30). Koch's postulates were satisfied by inoculating two rhododendron plants (cvs. PJM and Nova Zembla) with the putative pathogens. On each plant, each of three leaves was pierced with a dissecting needle and was inoculated by placing a 0.5-cm-diameter plug of mycelium that was taken from the margin of a colony actively growing on PARP-V8 agar on the wound. The inoculum was retained using clear adhesive tape. A similar procedure was used for twigs. Controls consisted of inoculations with sterile PARP-V8 agar medium. Both cultures of *P. inflata* and *P. insolita* produced necrotic lesions in all inoculations on both tissue types within 1 week, and they were reisolated from the margins of lesions on PARP-V8. The lesion margin was at least 2 cm away from the inoculum plug in leaf inoculations and several centimeters in twig inoculations. To our knowledge, this is the first report of *P. inflata* and *P. insolita* occurring on rhododendron and the first time *P. insolita* has been reported outside of Southeast Asia where it has been recovered only from soil.

Reference: (1) D. J. Stamps et al. Mycol. Pap. No. 162. CAB Int. Mycol. Inst. Wallingford, UK, 1990.