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# plant disease

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## Disease Notes

## First Report of Calonectria Leaf Spot Caused by *Calonectria colhounii* (Anamorph *Cylindrocladium colhounii*) on *Rhododendron* in Belgium

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Belgium is one of the most important *Rhododendron*-producing areas in Europe, with an annual sale of approximately 1.6 million plants. In June 2010, an outbreak of leaf spots several thousands of *Rhododendron* cv. Marcel Menard plants took place at a nursery near Gent. Diseased plants showed dark brown leaf spots that enlarged and finally resulted in leaf drop. Symptoms developed most explicitly on this cultivar, especially after standard repotting during May or June and when repotting was followed by a few days of unusually warm temperatures (30 to 35°C). The leading edge of diseased leaf tissue was excised, surface disinfected with 1% NaOCl for 60 s, and rinsed twice with sterile distilled water before being plated onto potato dextrose agar (PDA). After 5 days of incubation at 21°C in the dark, *Cylindrocladium*-like fungal colonies with white aerial mycelium and amber-brown growth within the agar consistently developed. Mycelium was transferred aseptically to fresh plates of PDA and incubated for 10 to 14 days at 17°C under a 12-h fluorescent light regimen to study the morphological characteristics. Conidiophores show

a penicillate arrangement of fertile branches, producing two to six phialides. They arose from a stipe and terminated in a clavate vesicle (3 to 5  $\mu\text{m}$ ). Conidia were straight, cylindrical, rounded at both ends, three septate, and measured 60 to 70  $\times$  4 to 6  $\mu\text{m}$ . Yellow subglobose to oval perithecia were abundantly produced. Asci were clavate, four spored, and measured 100 to 150  $\times$  15 to 30  $\mu\text{m}$ . Ascospores were hyaline, three septate and measured 50 to 65  $\times$  5 to 6  $\mu\text{m}$ . These characteristics are consistent with those of *Calonectria colhounii* Peerally (anamorph *Cylindrocladium colhounii*) (1). The  $\beta$ -tubulin gene was PCR-amplified with DNA extracted from the mycelium and the T1 and T2 primers (3), sequenced directly with a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA), and the DNA sequence was deposited (GenBank Accession FJF802784). BLASTn alignment showed 99% identity (525 of 526 nucleotides) with the  $\beta$ -tubulin DNA sequence derived from *Calonectria colhounii* CBS 293.79 (GenBank Accession No. DQ190564). A spore suspension ( $10^5$  conidia per ml) was prepared from 1-week-old culture, and 50- $\mu\text{l}$  drops were used to inoculate the abaxial side of 10 detached 1-year-old leaves from *Rhododendron* cv. Cunningham's White. Ten control leaves were inoculated with water. The leaves were placed in a moist chamber and incubated at 21 $^{\circ}\text{C}$  in the dark. After 5 to 6 days, all spore-inoculated leaves showed lesions identical to those on the naturally infected leaves, while the water-inoculated leaves remained symptom free. Following the original procedure, the fungus was reisolated from the diseased leaf and the morphological characteristics of the resulting culture were the same as those of the inoculated isolate, completing Koch's postulates. This fungus has been described on *Rhododendron* in the United States (2), but to our knowledge, this is the first record of *Calonectria colhounii* on *Rhododendron* in Belgium.

*References:* (1) P. W. Crous. Taxonomy and Pathology of *Cylindrocladium* (*Calonectria*) and Allied Genera. The American Phytopathological Society, St. Paul, MN, 2002. (2) P. W. Crous et al. Stud. Mycol. 55:213, 2006. (3) K. O'Donnell and E. Cigelnik. Mol. Phylogen. Evol. 7:103, 1997.

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