First Report of Stem Dieback and Leaf Spot of *Leucothoe* caused by *Cylindrocladium colhounii* in North Carolina

D. M. Benson and K. C. Parker, Department of Plant Pathology, and M. Munster, NCSU Plant Disease and Insect Clinic, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; and K. L. Ivors, Department of Plant Pathology, North Carolina State University, Mills River, NC 28759

Corresponding author: D. M. Benson. mike_benson@ncsu.edu


*Leucothoe fontanesiana* (*L. catesbaei*) and *L. axillaris* are common in the nursery trade for landscape use in USDA Zones 5 to 8. In the summer of 2009, one-year-old plants growing in 3-gal pots with a poorly-drained potting mix in a North Carolina wholesale nursery exhibited leaf spot and shoot dieback that resulted in over 50% mortality in some blocks of plants (Fig. 1). Isolations on acid PDA from stem and leaf tissue from plants submitted to the clinic resulted in recovery of isolates morphologically identical to *Cylindrocladium colhounii* Peerally (teleomorph *Calonectria colhounii* Peerally) (1) (Fig. 2A). This species is not reported on *Leucothoe* but found on many trees and shrubs worldwide including *Pinus strobus* in North Carolina, *Carya* sp. in Virginia, *Callistemon rigidus* in Florida, *Gaultheria procumbens* in Oregon, and *Ficus carica* in Louisiana (2). Morphological characters of the anamorph included 3-septate conidia roughly 58 to 63 µm long, and a clavate vesicle on the stipe (Fig 2B). The teleomorph was produced on host tissue after moist-chamber incubation (Fig. 3A). Perithecia were yellow, with the bases turning red when placed in 3% KOH. Ascospores were 3-septate, 49-58 × 5-7 µm (Fig. 3B). Isolates also were cultured from symptomatic plants of *L. axillaris*, *L. fontanesiana* ‘Girard’s Rainbow,’ and *L. fontanesiana* ‘Scarletta’ in 3-gal pots collected during a subsequent site visit. Both the internal transcribed spacer region (ITS1, 5.8S, and ITS2) of the ribosomal DNA (rDNA) and part of the β-tubulin gene of two isolates from the original clinic isolation, as well as three isolates collected from plants during the site visit, were sequenced to confirm fungal identification (1,3).

**Fig. 1.** Symptoms of *Cylindrocladium* leaf spot and stem dieback caused by *C. colhounii* on *Leucothoe fontanesiana* ‘Scarletta’ from the original specimen submitted to the NCSU Plant Disease and Insect Clinic.
Healthy plants with a new flush of growth in 1-gal pots in saucers were inoculated with one of four isolates (an original clinic isolate or one each from the three cultivars) in a greenhouse covered with shadecloth. At inoculation on 25 May 2010, two petri plates of each isolate were blended singly in 250 ml of deionized water, and then screened through several layers of cheesecloth. Fifty milliliters of an individual isolate suspension were sprinkled over the canopy of a test plant with a plastic bottle containing several 3-mm-diameter holes in the lid. Immediately after inoculation, the plants were double bagged: up around the saucer and pot, and then down over the foliage and lower bag. Bags were left in place for 2 days without further irrigation. Two plants of ‘Scarletta’ and one plant each of *L. axillaris* and ‘Girard’s Rainbow’ were inoculated per isolate, for a total of eight ‘Scarletta’ plants and four plants each of *L. axillaris* and ‘Girard’s Rainbow’ inoculated. One plant of each cultivar was bagged and left as a non-inoculated control. Plants were arranged in a completely randomized design on the greenhouse bench.

Necrotic leaf and stem symptoms developed over the next 3 weeks with all isolates causing disease. Leaf spots ranged from small individual lesions to larger zonate, necrotic areas that coalesced particularly along the leaf margin. Dieback progressed from stem tips a few centimeters down stems of the youngest growth on some plants (Fig. 4). Disease severity was rated 30 days post-inoculation for each plant on a 1 to 4 scale, where 1 was healthy foliage, 2 was necrosis on a few leaves, 3 was necrosis on many leaves, and 4 was necrosis on most leaves and stem dieback. Disease rating averaged 1.8 for *L. axillaris*, 2.5 for *L. fontanesiana* ‘Girard’s Rainbow,’ and 3.0 for *L. fontanesiana* ‘Scarletta’, respectively, for inoculated plants across the four *Cylindrocladium* isolates. Symptomatic tissue was collected for re-isolation on acid PDA and *C. colhounii* was recovered from every inoculated plant but not from the control plants. Sequences of the internal transcribed spacer region of the rDNA and the β-tubulin gene of twelve of the recovered isolates (four from each of the three cultivars) were identical to sequences of the four isolates used for inoculation. Representative sequences of our isolates have been deposited in GenBank.
This is the first report of *C. colhounii* attacking *L. axillaris* and *L. fontanesiana* in North Carolina.

Fig. 4. Symptoms of *Cylindrocladium* leaf spot and stem dieback caused by *C. colhounii* on *Leucothoe fontanesiana* ‘Scarletta’ 30 days after inoculation: (A) leaf spot and stem dieback in canopy; and (B) close-up of zonate leaf spot and dieback.

**Literature Cited**