

Diseases Caused by Nematodes

First Report of the Beech Leaf Disease Nematode *Litylenchus crenatae mccannii* (Nematoda: Anguinidae) in Michigan

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The North American beech leaf disease (BLD) nematode, *Litylenchus crenatae mccannii* Handoo, Li, Kantor, Bauchan, McCann, Gabriel, Yu, Reed, Koch, Martin and Burke, 2020, is recognized as a newly emergent nematode species that causes BLD in beech trees (*Fagus* spp.) in North America (Carta et al. 2020; Kantor et al. 2022a). Since the first report of BLD on American beech (*Fagus grandifolia* Ehrh) within Lake County, located in the northeastern corner of the state of Ohio in 2012 (Carta et al. 2020), the disease has rapidly spread to other U.S. states and a province in Canada (Carta et al. 2020; Ewing et al. 2018; Kantor et al. 2022b; Marra and LaMondia 2020; Reed et al. 2020). Currently, besides Ohio, this nematode has been reported in Pennsylvania, New York, Connecticut, Massachusetts, Maine, Rhode Island, New Jersey, West Virginia, and Virginia, as well as Ontario, Canada. Different life stages of *L. crenatae mccannii* were isolated from symptomatic American beech leaves from an isolated natural maple-beech stand in rural Saint Clair County, Michigan, U.S.A., presenting typical symptoms of beech leaf disease, i.e., swelling and darkening of interveinal leaf tissues. Samples were taken to the Forest

Pathology Laboratory at Michigan State University where the presence of *L. crenatae mccannii* was confirmed in the leaves, after which samples were sent to the Mycology and Nematology Genetic Diversity and Biology Laboratory (USDA-ARS) in Beltsville, Maryland, for official confirmation. Nematodes were identified based on morphology and sequence analysis of the internal transcribed spacer (ITS), and the D2D3 region of the 28S large subunit ribosomal DNA. To validate the morphological identification, two different ribosomal DNA *loci* were amplified and sequenced and the phylogenetic relationships were generated. The amplification yielded fragments of 784 and 741 bp flanked by the ITS (GenBank accession no. OP689654) and D2D3 (GenBank accession no. OP689710) primers, respectively. The sequences obtained for the specimens collected in Michigan revealed 100% similarity to *L. crenatae mccannii* sequences obtained from specimens collected from other geographical areas in the United States, validating the morphological analyses. The ITS sequence shared a 99.75% similarity with the subspecies *L. crenatae* (GenBank accession no. LC383724.1), and 90.53% similarity to *L. coprosma* Zhao, Davies, Alexander and Riley, 2011 (GU727548.1). While the D2D3 sequences of both *L. crenatae* subspecies revealed a 100% similarity (versus LC383725.1), they revealed 95.35% similarity to *L. coprosma* (KY679564.1). Since the first confirmed detection of BLD in June 2022 in St. Clair County, BLD has been reported in Oakland and Wayne counties (seven reports total across the three counties), suggesting BLD spread in the southeast of Michigan. BLD confirmation was based on either physical symptoms (leaf banding), and/or the presence of the beech leaf nematode by morphological or molecular confirmation. The presence of the beech leaf nematode in symptomatic leaves follow the results obtained by Carta et al. (2020) from inoculation of beech seedlings with *L. crenatae mccannii*. Based on both morphological and molecular analyses, the specimens collected in Michigan were identified as *L. crenatae mccannii*. To our knowledge, this is the first report of this species in conjunction with symptomatic *F. grandifolia* leaves in this state.

References:

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