

## **Microscopy and organismal associations of *Litylenchus crenatae* in beech leaf disease of *Fagus grandifolia* in Ohio, USA.**

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American beech trees are a major component of many deciduous forest areas in eastern North America, and a new disease was discovered affecting beech near Cleveland, Ohio in 2012. Beech leaf disease (BLD) caused striped, galled leaf tissue, canopy thinning and sometimes young tree mortality. Scientists from the US and Canada demonstrated that invasive *Litylenchus crenatae* nematodes from diseased leaves could cause BLD symptoms on seedlings primarily through bud inoculation. Low-temperature scanning electron microscopy images of the nematodes from symptomatic bud and leaf tissue revealed that nematodes filled spaces around spongy parenchyma. Nematodes entwined around the bodies of mites were imaged, and an inventory of mites was made at the Holden Arboretum near Cleveland. Bird mites were also collected and imaged because a subtractive molecular microbiome survey showed different bacterial communities between symptomatic and asymptomatic leaves, including the presence of the intracellular endosymbiont *Wolbachia* on symptomatic leaves. Recovered *Wolbachia* sequences showed high affinity to bacteria isolated from a quill mite associate of the rose finch family of birds. One finch is known to consume beech buds in North America but not in Asia. Birds, mites and *Wolbachia* may be important for nematode distribution and disease etiology. The disease is spreading primarily eastward and identifying agents of transmission is critical to stop BLD progression by foresters and plant pathologists developing integrated controls against BLD.

**Keywords:** Anguinata - Fagaceae - Invasive species - Disease transmission - Scanning electron microscopy.