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DISEASE NOTES

First Report of Geosmithia morbida on Ambrosia Beetles Emerged From Thousand Cankers-diseased Juglans nigra in Ohio

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Eastern black walnut (*Juglans nigra*) is a highly-valued species for timber and nut production in the eastern United States. Thousand cankers disease (TCD), caused by the interaction of the walnut twig beetle (*Pityophthorus juglandis*) and the canker fungus*Geosmithia morbida* (Tisserat et al. 2009), was first found in the eastern United States in 2010 and is a threat to the health of black walnut in its native range. The recent detection of *G. morbida* on a weevil species (*Stenomimus pallidus*) (Juzwik et al. 2015) led to an effort to determine the extent to which beetles other than *P. juglandis* acquire the fungus on their bodies from TCD-symptomatic trees. Four TCD-symptomatic *J. nigra* (20 to 23 cm diameter at 1.4 m stem height) on two adjacent residential lots in Hamilton, OH, were felled on 9 September 2014. Eight main stem and eight branch sections (30 cm long) from each tree were placed in insect-rearing buckets. Ambrosia and bark beetles and weevils that emerged were collected weekly for 3 months, identified, and stored singly in 1.5-ml microcentrifuge tubes at –20 C. Fungal assays for *G. morbida* were conducted on the collected specimens using a dual assay procedure involving maceration of each insect in 40 µl molecular grade water. Half of each suspension was subjected to serial dilution plating (SDP) on ¼-strength potato dextrose agar amended with chloramphenicol and streptomycin and the other half used for fungal DNA extraction, polymerase chain reaction (PCR), and DNA sequencing (molecular assay) as per Juzwik et al. 2015. Putative *G. morbida* isolates obtained from SDP plates were confirmed by DNA sequencing. A *G. morbida*-specific primer (3'-CGACCCGGACCCAGGCGACCG-5') was paired with ITS4 for DNA amplification in the molecular assay and for fungal isolate identification. Eight Curculionidae species were obtained from only main stem sections of two trees. Two ambrosia beetles (*Xylosandrus crassiusculus* and *Xyleborinus saxeseni*) and one weevil species (*S. pallidus*) accounted for 149 of 155 collected specimens. *G. morbida* was only detected on these three species. Frequency of fungus detection differed by assay method and insect species. *Geosmithia morbida* was most commonly detected on *X. crassiusculus*(*n* = 26) (10, SDP; 15, molecular) with 17 of the specimens being *G. morbida*-positive based on composited results. Detection levels were less for *X. saxeseni* (assayed 68 of 76 collected) (7, SDP; 13, molecular) with 15 positive adults based on composited results. For*S. pallidus* (*n* = 47), a higher number yielded *G. morbida* using SDP (10) versus molecular assay (4) with 13 *G. morbida*-positive weevils found based on composited results. Sequences obtained from SDP and molecular assays had ≥ 99% identities to multiple accessions for *G. morbida* in GenBank. To our knowledge, this is the first report of *G. morbida* on ambrosia beetle species. The detection on the *S. pallidus* complements the fungus' recent find on specimens emerged from girdled walnut trees in Brown Co., IN. We hypothesize

that *G. morbida*-laden ambrosia beetles may exacerbate TCD symptom progression in areas with the disease. The role of all three insect species in the epidemiology of TCD requires further study.

References

Juzwik, J., et al. 2015. Plant Health Prog. 10.1094/PHP-RS-14-0030.

Tisserat, N., et al. 2009. Plant Health Prog. 10.1094/PHP-2009-0811-01-RS.