First Report of Bacterial Leaf Spot Caused by *Xanthomonas arboricola* Infecting Saucer Magnolia in Tennessee

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Saucer magnolia (*Magnolia* x *soulangeana*) is a deciduous tree with early blooming flowers and widely planted in the south of England and western and eastern United States. Saucer magnolia plants in 5 gal containers with symptoms of leaf spot were received from a commercial nursery in DeKalb Co., TN in July 2015. The symptom was dark brown-black, angular necrotic lesions with yellow halos. The disease severity (percentage leaf area diseased) was nearly 35% and the disease incidence was nearly 80% of 10,000 plants. Bacterial streaming was observed microscopically from necrotic leaf tissues. Bacteria were isolated from surface-sterilized infected leaf tissue (0.525% sodium hypochlorite; 1 min) by plating 10- fold serial dilutions onto yeast dextrose carbonate (YDC) and Pseudomonas F agar media. Yellow, mucoid colonies were obtained consistently from symptomatic leaves plated onto YDC. No other bacteria were isolated. Bacteria were gram negative, oxidase and arginine dihyrolase activity were negative, catalase, levan, esculin and gelatine hydrolysis were positive, and growth occurred at 35°C. Infiltration of tobacco leaves with bacterial suspensions of the magnolia strains (FBGM-1 and FBGM-2) resulted in typical hypersensitivity reactions within 24 h. Four symptomatic leaves and two purified strains

were tested using the *Xanthomonas* immunoblot test (Agdia, Inc., Elkhart, IN). All of the samples were positive. To confirm pathogen identity, total genomic DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) directly from a 2-day old culture (FBGM-1). The 16S rRNA, chaperone protein dnaK (*dnaK*), ton B-dependent receptor (*fyuA*), DNA gyrase subunit B (*gyrB*) and RNA polymerase sigma factor (*rpoD*) genes were amplified and sequenced. The primer sets used in this study were 8F/1492R and fD1/rD1 (16S rRNA) (Galkiewicz and Kellogg, 2008; Weisburg et al. 2012), XdnaK1F/XdnaK1R (*dnaK*), XfyuA1F/XfyuA1R (*fyuA*), XgyrB1F/XgyrB1R (*gyrB*), and XrpoD1F/XrpoD1R (*rpoD*) (Young et al. 2008). The sequences (GenBank acc. nos. MT210900, MT210904, MT226277, MT226275, MT226273, and MT226271) were 98.6-100% similar to those of *Xanthomonas arboricola* strains from several hosts in multiple countries in the NCBI database (GenBank acc. nos. LC388645, AB911210, KX357120, KT874177, KT886371, and EU499159, respectively). Pathogenicity test was performed once using isolated bacterial strain (FBGM-1) on containerized saucer magnolia plants. Three plants were sprayed with a bacterial suspension (10^8 CFU/ml), covered with clear plastic for 24 h, and directly placed into a greenhouse at 25 to 30°C. Control plants were sprayed with sterilized water and kept in the same condition. Inoculated plants showed leaf spot symptoms after 2 weeks. The bacterium was re-isolated from leaf spots as described above. All control plants remained symptom-free and the bacterium was not isolated from leaf tissue. Bacterial leaf spot of magnolia, caused by bacterium belong to *Xanthomonas* genus, was reported in commercial nursery production in Florida in 2012 (Knox et al., 2012). Xanthomonads isolated in New Zealand from diverse hosts, including *Magnolia*, were classified as *X. arboricola* (Young et al.

2010). To the best of our knowledge, this is the first report of bacterial leaf spot on saucer magnolia caused by *X. arboricola* in Tennessee. Overhead irrigation system likely contributed to the outbreak of this disease. For the near future, timely bactericide applications will be necessary to manage this disease on saucer magnolia in affected nurseries.

References:

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