Diseases Caused by Bacteria and Phytoplasmas

First Report of *Richardia scabra* as a Symptomatic Host of ‘*Candidatus Phytoplasma trifolii*’ (16SrVI-A Subgroup) from Bengaluru, India

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The rubiaceous plant Florida pusley (*Richardia scabra* L.) is a naturalized annual weed found in most parts of India. During 2012 to 2018, *R. scabra* plants exhibiting phytoplasma disease-like symptoms such as stunting, malformed inflorescence, virescence, phyllody, and undersized leaves were found on the National Bureau of Agricultural Insect Resources Research Farm in Bengaluru as well as in neighboring fields, with a mean incidence ranging from 5 to 8%. Phytoplasma was the suspected cause of disease, because usually eggplant (*Solanum melongena* L.) and sesame (*Sesamum indicum* L.) crops—both of which suffer from phytoplasma-induced diseases—are raised for experimental purposes in that area. Negative sap-inoculation results from 10 symptomatic plants in a screenhouse experiment ruled out the involvement of a mechanically transmitted pathogen (e.g., a virus). The disease agent could be transmitted to four out of 10 healthy plants through grafting, reproducing symptoms that were milder than those in the field. No known leafhopper or planthopper vector was constantly associated with the plant, except for sporadic probing visits by adults of *Hishimonus phycitis* (Distant), a known phytoplasma vector. Adult *H. phycitis* preferred eggplant or sesame rather than *R. scabra* in choice tests, but when confined, the leafhopper could transmit the pathogen to 43% of plants. Genomic DNA was isolated from

aboveground parts of three field-collected symptomatic plants. Targeting the phytoplasma *16S rDNA* gene, DNA was amplified with universal primers P1/P7 and R16F2n/R16R2 in primary (Deng and Hiruki 1991; Schneider et al. 1995) and nested PCR (Gundersen and Lee 1996; Lee et al. 1993), respectively. DNA from three asymptomatic (apparently healthy) plants served as the negative control. The ~1.2-kb amplified product of *16S rRNA* gene of phytoplasma DNA was detected in all the three symptomatic plants but not in asymptomatic control plants. PCR products were gel-eluted using a gel extraction kit and sequenced with an ABI 3500 xL Genetic Analyzer using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). The nucleotide sequence deposited in NCBI GenBank (accession no. MN663123) is closest (100% identity) to ‘*Candidatus Phytoplasma trifolii*’ isolate Tirupati (accession no. KP899062) in BLAST. The virtual restriction fragment length polymorphism pattern derived through *iPhyClassifier* (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>; Zhao et al. 2009) indicated that the sequence is closest to the reference pattern of the 16SrVI-D subgroup (GenBank accession no. X83431). To the best of the author’s knowledge, this is the first report of a phytoplasma-associated disease in *R. scabra*. Because *H. phycitis* was noticed to visit *R. scabra*, and because eggplant and sesame are also hosts of the same leafhopper, farmers need to keep their fields free from this weed.

References:

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