

Disease Notes

First Report of '*Candidatus Liberibacter asiaticus*' Associated with Huanglongbing in Sweet Orange in Ethiopia

M. Saponari, Institute of Plant Virology, CNR, Bari, Italy; **G. De Bac** and **J. Breithaupt**, FAO Plant Protection Office, Rome, Italy; **G. Loconsole**, Department of Plant Protection and Applied Microbiology, University of Bari, Italy; **R. K. Yokomi**, United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Parlier, CA; and **L. Catalano**, Consorzio Vivaistico Pugliese, Bari, Italy

Huanglongbing (HLB) is a serious disease of citrus worldwide. Three different '*Candidatus Liberibacter*' species are associated with HLB: '*Ca. Liberibacter asiaticus*', '*Ca. L. africanus*', and '*Ca. L. americanus*' (1). '*Ca. L. africanus*' and its vector, *Trioza erytreae*, are both heat sensitive, and when present, occur in citrus when temperatures remain below 30 to 32° C. In Africa, '*Ca. L. africanus*' and *T. erytreae* have been reported in South Africa, Zimbabwe, Malawi, Burundi, Kenya, Somalia, Ethiopia, Cameroon, and Madagascar (1). Inspection of citrus trees in orchards and budwood sources in nurseries located in the warmer citrus-growing areas of Tigray and North Wollo in northern Ethiopia revealed nearly 100 trees with symptoms of leaf yellowing with a blotchy mottle pattern, dead branches, and decreased fruit quality and yield. Two symptomatic sweet orange budwood trees and three symptomatic orchard plants were sampled in April 2009, along with three healthy-looking sweet orange plants. DNA was extracted from 200 mg of desiccated leaf midribs using the CTAB method (4) and subjected to conventional PCR using the primer pairs A2/J5 (2) and O12/23S1 (3) that amplify the ribosomal protein gene in the rplKAJL-rpoBC operon and the 16S/23S ribosomal intergenic regions, respectively, of '*Ca. L. africanus*' and '*Ca. L. asiaticus*'. Positive PCR reactions were obtained for all five symptomatic samples with both primer pairs. PCR amplicons of 703 bp (A2/J5) and 892 bp (O12/23S) recovered from two of these samples were purified, cloned, and sequenced. BLAST analysis revealed that the nucleotide sequences we obtained for the ribosomal protein (GenBank Accessions Nos. GQ890155 and GQ890156) shared 100% identity with each other and 99% identity with sequences of '*Ca. L. asiaticus*' from Brazil (DQ471904), Indonesia (AB480161), China (DQ157277), and Florida (CP001677). Similarly, the 16S/23S ribosomal intergenic sequences (GU296538 and GU296539) shared 100% identity with each other and 99% identity with homologous '*Ca. L. asiaticus*' sequences from Brazil (DQ471903), Indonesia (AB480102), China (DQ778016), and Florida (CP001677) and contained two tRNA genes as occurs in '*Ca. L. asiaticus*' but not in '*Ca. L. africanus*' (3). To our

knowledge, this is the first report of '*Ca. L. asiaticus*' in Africa. The presence of '*Ca. L. asiaticus*' is a threat for warmer citrus-growing areas of Africa that are less favorable for '*Ca. L. africanus*' and *T. erythraeae*. In areas where '*Ca. L. asiaticus*' was confirmed, symptomatic trees must be promptly eradicated and surveys to determine spread of the disease and its vectors are necessary.

References: (1) J. M. Bove. J. Plant Pathol. 88:7, 2006. (2) A. Hocquellet et al. Mol. Cell. Probes 13:373, 1999. (3) S. Jagoueix et al. Int. J. Syst. Bacteriol. 47:224, 1997. (4) M. G. Murray and W. F Thompson. Nucleic Acids Res. 8:4321, 1980.