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# plant disease

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## Disease Notes

### First Report of *Candidatus Liberibacter asiaticus* Associated with Citrus Huanglongbing in California

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Huanglongbing (HLB), also known as citrus greening, is one of the most destructive citrus diseases worldwide and is seen as a major threat to the multimillion dollar citrus industry in California. The vector of the two bacterial species associated with this disease, *Candidatus Liberibacter asiaticus* and *Ca. L. americanus*, is the Asian citrus psyllid (ACP), *Diaphorina citri* (4). ACP was detected in California in August of 2008 and has since been detected in nine counties in southern California. As part of a long term survey and testing program for the ACP carrying the HLB associated bacteria, groups of ACP nymphs and adults were submitted to the Jerry Dimitman Citrus Research Board/Citrus Pest and Disease Prevention Program Laboratory in Riverside, CA. In March 2012, DNA extracted using the Qiagen MagAttract 96 DNA plant kit (QIAGEN Inc., 27220 Tumberry Lane, Suite 200, Valencia, CA 91355) from a group of three ACP adults tested positive for *Ca. L. asiaticus* with the real-time PCR assay developed by Li et al. (4). ACP adults were collected from a residential citrus tree located in the Hacienda Heights area of Los Angeles County, California. The approximately 1.8 meter tall lemon tree had 23 graft unions, primarily of lemon (*Citrus × meyeri*) and pomelo (*Citrus maxima*) varieties. The tree was unthrifty, with yellow shoots and chlorotic leaves. Symptoms on the lemon and pomelo leaves included asymmetrical blotchy mottling, yellowing, and corking of the leaf veins, with the blotchy mottle more prominent in the pomelo leaves. Pomelo

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leaves appeared crinkled along the thickened veins. Lemon leaves had yellow veins and a few had islands of green tissue completely surrounded by yellow tissue. The entire tree was removed, cut into sections, bagged, and transported to the CDFA Plant Pest Diagnostics Lab for analysis. Two hundred milligrams of petiole and midrib tissue from leaves apical to each graft union was collected, and DNA from each sample was extracted using the Qiagen DNeasy plant mini kit. DNA extracted from both lemon and pomelo leaves tested positive for *Ca. L. asiaticus* using real-time PCR (4). A 1,160-bp fragment of the 16S ribosomal RNA gene was amplified from the insect and plant DNA extracts using conventional PCR with primers OI1 and OI2c (2). A 703-bp fragment of the  $\beta$ -operon gene was amplified from the insect and plant extracts with primers A2 and J5 (1). The 16S rDNA fragments from the insect and plant respectively (GenBank Accession Nos. JX430434 and JX455745) and the  $\beta$ -operon fragments (JX430435 and JX455746) showed 100% identity with the corresponding regions of *Ca. L. asiaticus* (CP001677) strain psy 62. Our 16S rDNA sequence showed 98% identity with *Ca. L. africanus* (EU921620), 97% identity with *Ca. L. solanacearum* (HM246509), and 96% with *Ca. L. americanus* (FJ036892). In response to the detection of HLB, a 241 km<sup>2</sup> quarantine area around the detection site was established. Surveys for ACP and symptomatic host plants within the HLB quarantine area are ongoing. To date, there have been no additional positive detections. In the United States, HLB was first detected in Florida in 2005 (4) and in Texas in January of 2012 (3). To our knowledge, this is the first confirmed report of *Ca. L. asiaticus* associated with HLB in California.

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