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5.1 Liberibacter Populations in Citrus and Orange Jasmine Trees in the State of São Paulo, Brazil

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Two pathogens are associated with huanglongbing in Brazil: '*Candidatus* L. americanus' (Lam) and '*Ca.* L. asiaticus' (Las). Both are transmitted by the Asian citrus psyllid *Diaphorina citri* Kuwayama, first reported in Brazil in 1942 (2), and differ in their sensitivity to temperatures of 32°C and above, in graft transmission efficiency, and in their ability to multiply in citrus (Lopes et al., submitted). This helps to explain their uneven spatial progress and the shift in bacterial prevalence in the State of São Paulo State (SPS) over time. Hosts of Liberibacter in SPS include all commercial citrus and the ornamental orange jasmine (*Murraya paniculata*) (3,4), which is very common in most Brazilian cities. Surveys to determine the extent of Liberibacter occurrence in orange jasmine have been carried out since 2004 in the streets of 21 localities in the center of SPS. Randomly chosen old trees with symptomatic yellow shoots whether or not associated with shoot dieback were sampled. These symptoms were similar to those observed when the pathogen was first detected in orange jasmine (3). Lam and Las have been detected in 11.4% and 0.5%, respectively, of 550 sampled trees. Lam was detected in eight localities (Água Vermelha, Américo Brasiliense, Araraquara, Bueno de Andrada, Matão, Motuca, Santa Lúcia, Silvânia), and Las at two localities (Santa Eudóxia and Araraquara). The higher incidence and spatial distribution of Lam in orange jasmine might be related to the period of time Lam has predominated in those areas. On the basis of grower information and disease incidence levels observed in 2004 in the first affected citrus farms, it was estimated that HLB was already present in the center of SPS, 9 to 10 years before the first report (1,5). Field surveys and data from the Fundecitrus diagnostic laboratory indicate that Lam was the most prevalent, or may be the only Liberibacter species present, in that area at that time. Therefore, Lam might have had sufficient time to move from citrus to orange jasmine, and vice-versa, without any of the limitations provided by removal of symptomatic trees or applications of insecticides, practices currently adopted in the management of HLB in citrus orchards. In the last 4 years, a disproportional increase in Las incidence was, however, observed in citrus trees in SPS.

Whether this phenomenon also applies to Las in orange jasmine is not known at this point. Recently, DNAs extracted from leaf midribs of most infected orange jasmine sampled from 2004 to 2008, and from leaf midribs of a set of 116 infected citrus trees sampled by growers in the same time period and sent to Fundecitrus for diagnostic purposes through the use of conventional PCR, were further analyzed by quantitative real-time PCR (qPCR). Average cycle threshold values for Lam and Las were, respectively, 32.69 (n 53, SE 0.27) and 32.46 (n 5, SE 0.99) in orange jasmine, and 26.82 (n 58, SE 0.17) and 25.12 (n 58, SE 0.28) in citrus trees. The equivalent number of Liberibacter cells in 50 ng total DNA used per qPCR was, on average, for Lam and Las, respectively, 23 and 218 in orange jasmine, and 995 and 27,959 in citrus. The number of orange jasmine trees so far found to be affected by Las and included in this study was, however, too low for data comparison. Also, Liberibacter cell titers were assessed in naturally infected field-grown trees. The time intervals between inoculation and sampling dates were, therefore, not known. Insect transmission experiments, currently in progress in potted grown-

trees, should bring more information on this matter and on the importance of orange jasmine for the HLB epidemics.

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