3.10 PCR for detection of Asian and American strains of Candidatus Liberibacter in Citrus, Murraya and Diaphorina from Northwest Argentina

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Huanglongbing (HLB) (syn= citrus greening) was detected in São Paulo, Brazil, in September, 2004, and in Florida, USA, one year later in September 2005. In Brazil the disease is associated with the phloem-limited bacteria Candidatus Liberibacter asiaticus (Las) and Ca. L. americanus (Lam) and can be transmitted by graft and by the Asian citrus pysllid, Diaphorina citri. HLB is considered the most destructive citrus disease in the world. There are no commercial varieties known to be tolerant to the disease and no effective control method. The symptoms of HLB on leaves are blotchy mottle, corky veins and asymmetric yellowing, and on fruits, colour inversion, size reduction and fruit drop. All of these symptoms are characteristic but not specific for HLB. The disease has not been detected in Argentina but is present near the eastern border with Brazil in Parana State (Altonia). The insect vector Diaphorina citri, has been present in the northeastern citrus region of Argentina since 1984 and in the extreme northwestern region since 2006. Murraya paniculata, a weakly symptomatic host for HLB, is widely planted as an ornamental plant in both regions. Highly sensitive and specific detection tools are needed for early and accurate detection of the pathogen. The objective of this program is to adopt an accurate PCR methodology for identification of Las and Lam in plants and in psyllids. In 2005 we implemented a Duplex PCR technique employing GB1 and GB3, primers that amplify a 1027 bp region from the 16sRNA for Las, (Teixeira et al 2005) and a 703 bp region from the nusG-rp/Kajl-rpoBC operon for Lam (Hocquellet et al, 1999). Modifications in protocols were made to accommodate the PCR cyclers and to adjust for the small size of tissue DNA samples. Later, the primers of Hung et al. (1999) were employed for Las which are more sensitive than the those of Teixera et al. (2005) and Li et al (2007).

Different extraction methods were tested for Citrus, Murraya and insect tissues to optimize the amount of plant or insect tissues (CTAB- Murraya); (Hung, et al., 1999 ; M. Irey, personal communication). Universal extraction bags yielded better results than hand maceration. Nested PCR was also trialed to improve the detection sensitivity to 0.01 ng of infected DNA (Texeira et al, 2008). The positive control was Candidatus species DNA from Brazil and USA. For Diaphorina samples, DNA from infected psyllids provided by Brazil was employed. Samples with suspicious symptoms of HLB were from orchards located in Salta and Jujuy provinces where Diaphorina citri is present. Thus far, all samples have been negative. At present the EEAOC utilizes conventional PCR and nested PCR to detect the bacteria and a key group of researchers, technicians and field workers have been trained to recognize HLB symptoms and the vector.

References