

Detection of the “*Candidatus Liberibacter americanus*” in Phloem Vessels of Experimentally Infected *Catharanthus roseus* by Scanning Electron Microscopy

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RESUMO

Deteção de *Candidatus Liberibacter americanus* em vasos de floema de *Catharanthus roseus* infectados experimentalmente, utilizando microscopia eletrônica de varredura

“*Candidatus Liberibacter americanus*”, agente causal do Huanglongbing (HLB) foi visualizado pela primeira vez ao microscópio eletrônico de varredura infectando vasos do floema de plantas de vinca (*Catharanthus roseus*) experimentalmente infectadas utilizando-se *Cuscuta* sp. no Brasil.

Presence of Huanglongbing (HLB) disease in citrus orchards in the state of São Paulo has been known since 2004. Its identification was made by molecular techniques which revealed the presence of two forms of the bacteria “*Candidatus Liberibacter*”. One previously described in Asia as *Ca. L. asiaticus* and the other a novel type, *Ca. L. americanus* (Coletta-Fo *et al.*, Plant Dis. 88:1382. 2004; Teixeira *et al.*, Plant Dis. 89:107. 2005). Transmission electron microscopy confirmed the presence of characteristic bacteria in the phloem vessels of affected sweet orange leaves (Tanaka *et al.*, Fitopatol. Bras. 31:99. 2006). Attempts to observe these bacteria by scanning electron microscopy (SEM) to understand their three dimensional morphology were hindered by the extremely low concentration at which they usually occur in citrus plants. On the other hand, *Ca. L. americanus* was successfully transferred to the ornamental periwinkle (*Catharanthus roseus* (L.) G. Don.) using dodder (*Cuscuta* sp.) as previously reported for other *Ca. Liberibacter* (Garnier & Bové, Proc. Conf. IOCV 12: 212. 1993). Infected plants developed chlorotic symptoms and contained higher concentrations of the bacterium as evaluated by molecular techniques and transmission electron microscopy. For *in situ* observations of *Ca. L. americanus* under SEM, midribs from symptomatic leaves and young stems were fixed with aldehyde, infiltrated with glycerol, immersed in liquid nitrogen and fractured with a scalpel, and the pieces post-fixed in 0.1% OsO₄ for 24 h, dehydrated in acetone, dried at the critical point, gold coated by sputtering and examined in a LEO 435 VP scanning electron microscope. In the vascular region of the infected leaves and stems, exposed by the fracture of frozen tissues, many phloem vessels contained bacterial cells, interpreted as *Ca. L. americanus*, in varied numbers. Some vessels were obliterated by aggregated bacterial cells (Fig. 1 A). Bacteria were typically bacilliform and elongated, 2-3 μm long with rounded ends, 200-300 nm wide and with a smooth outer surface (Fig. 1 B). These values match those obtained in

sectioned bacterial cells. Such bacterial cells were absent in the phloem vessels of healthy, uninoculated periwinkle plants. This report is the first description of *Candidatus Liberibacter* by scanning electron microscopy.

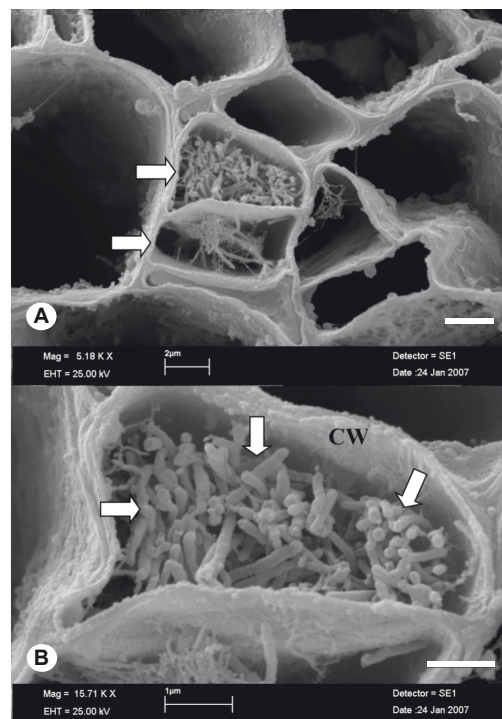


FIG. 1 – A. Scanning electron micrograph of phloem vessels (arrows) in cross section in a frozen-fractured leaf midvein of a periwinkle (*Catharanthus roseus*) experimentally infected by *Ca. Liberibacter americanus* using dodder. One of the vessels is filled by bacilliform cells of *Ca. L. americanus* (bar = 2 μm). B. Detail of A, showing the bacterial cells (arrows) within the phloem element (bar = 1 μm).

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