

Utilizing Real-Time qPCR and Scanning Electron Microscopy to Gauge Proliferation of Citrus Greening Disease in Persian Lime Trees in South Florida

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Citrus greening disease (HLB), first identified in 2005 in Miami-Dade, is caused by a fastidious, phloem-specific bacteria known as *Candidatus Liberibacter asiaticus* (CLAs), occurring in most species of citrus. The bacterium is transmitted by the Asian Citrus Psyllid, a mosquito-like vector. Symptoms of this disease include leaf mottling/yellowing, and fruit drop. The goal of this research was to examine the presence and location of CLAs in the vasculature of citrus leaves, and determine the appropriateness of various detection methods. Leaves from Persian lime were selected randomly from two groves. Real-time qPCR using a 96-well plate was employed to quantify the relative abundance of several reference genes from CLAs DNA in the leaf tissue (prophage repeat, 16S rDNA, EF-Ts, and L12P), suggesting presence of the infection. Then, scanning electron microscopy (SEM) was used to qualitatively describe results indicated by qPCR using images. SEM allowed for direct visualization of bacteria in phloem tissue. Upon analysis of two methods of disease detection - qPCR and SEM - it was observed that qPCR indicated moderate to high relative gene expression in samples from both locations, and images from SEM displayed evident bacteria presence in phloem tissue. This observation substantiates existing literature on the claim that CLAs is found solely in phloem. In combination, these two methods of gauging bacterial proliferation increased accuracy of detecting citrus greening disease and could establish a precedent for diagnosis of plagued citrus groves. This data will serve as a foundation for translational research in developing a cost-effective, phloem-selective therapeutic or in breeding cultivars of citrus that are physiologically fit against citrus greening disease.