8.20 Visualization of Ca. Liberibacter asiaticus in Immature Citrus Seed Coats by Fluorescent *In Situ* Hybridization (FISH) of 16S rRNA

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The huanglongbing disease of citrus is associated with infection by the non-cultivable bacterium *Ca. Liberibacter asiaticus*. During the course of a study on seed transmission in sweet orange and grapefruit, *Ca. Liberibacter asiaticus* DNA was detected in nucleic acid extracts from seed coats but not from embryos using real-time and conventional PCR. Low crossover threshold values (Ct) from real-time PCR assays suggested large numbers of bacteria were present in the intact seed coats. As PCR does not distinguish if bacterial DNA is derived from live or dead cells and as this bacterium is non-cultivable, FISH specific for 16S rRNA was applied to thin sections of immature grapefruit seeds to provide indirect evidence of the presence and the viability of bacterial cells. Fluorescence is expected from bacterial cells which have a high concentration of ribosomes so that the copy number of the 16S rRNA is 10,000 to 100,000 per cell, indicating high levels of protein synthesis and hence viable, metabolically active cells. In August 2010, immature seeds were collected from fruit from an infected Conner’s grapefruit tree, fixed and imbedded in paraffin. Intense fluorescence was observed from phloem cells within the vascular bundle and individual bacterial cells were observed in large numbers. This data suggests that, at least in immature seeds, large numbers of viable cells are present in the seed coat, but not in the embryo.