12.13 Delivery of antibacterial peptides into commercial citrus cultivars for the control of citrus greening (Huanglongbing or HLB) in Florida.

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Citrus greening (Huanglongbing, HLB) is the single most economically important disease among citrus diseases worldwide. The causal agent of this disease is thought to be the non-cultured, phloem-limited, α-proteobacterium, Candidatus Liberibacter. Three species of “Ca Liberibacter” have been associated with citrus greening: “Ca Liberibacter asiaticus” (Las), is predominant in Asia and Americas (mainly Florida and Brazil) transmitted by psyllid, Diaphorina citri Kuwayama; “Ca. Liberibacter africanus” in Africa, transmitted by Trioza erytreae; and in Brazil, another species, “Ca. Liberibacter americanus” (Lam). Since its first demonstration in August of 2005, HLB has spread rapidly into all major citrus growing regions in Florida. At this time, the control methods for HLB are reducing psyllid populations and removal of the infected trees, which may not be sustainable. In the long term, the most sustainable approach to manage citrus greening is to develop resistance in commercial cultivars. One approach is to express antibacterial peptides (AMP) in commercial citrus varieties to control the HLB bacteria. The AMP's, usually 20-40 amino acids long, are ubiquitous among eukaryotes and elicit innate defense against bacterial infections. The AMP’s could be engineered to express in citrus through transgenic technology or using viral vectors. We have used a citrus tristeza virus (CTV) vector to deliver AMP’s into sweet orange and grapefruit cultivars and subsequently challenge them with HLB to study the efficacy of AMP’s to mitigate HLB infection. Twenty to thirty different AMP’s of plant and animal origin were cloned with or without an export leader sequence into the CTV vector (Folimonov et al., 2007) between the CPm and CP genes behind the beet yellows virus CP subgenomic RNA controller element. These constructs were used to transfec Nicotiana benthamiana mesophyll protoplasts and the progeny virions were used to inoculate citrus plants. The result was the systemic spread of the virus and expression of the AMP throughout the trees. Details of the AMP’s, replication in protoplasts, and agro-inoculations will be presented.