

- (1) Continue screening of chemical compounds that eliminate or suppress the Las bacteria in periwinkle using the optimized regeneration system. The other chemicals, Metronidazole and ortho-phenylphenol, were tested for their ability to eliminate or suppress Las bacteria and to promote the growth of severe HLB-affected cuttings using the optimized regeneration system. The results showed that more than 2.0% of Metronidazole and ortho-phenylphenol significantly improved periwinkle cutting regeneration. The cuttings treated with less than 1% of metroidazole also regenerated very well. The Las-titers of the regenerated plants will be tested by quantitative real-time PCR.
- (2) When PS was foliar-sprayed once a week for three consecutive weeks on the Las-infected periwinkle plants at different rates (1x, 5x and 10x), no significant differences were found among treatment rates, but each treatment reduced the Las-bacteria to undetectable levels in the infected plants as compared to the water control. DMSO or Silwet L-7 as emulsifiers had no effect on Las-infected periwinkle plants.
- (3) HLB-affected citrus was foliar-sprayed or soaked in PS solutions once a week for three consecutive weeks in the greenhouse. The dynamics of Las bacteria were tested by PCR and Q-PCR monthly for a period of six months to determine if the PS affects the bacteria in citrus. After six months the Las-infected, PS treated citrus tested negative for Las by nested PCR, and undetected by real-time Q-PCR, but oxytetracycline and water control treatments were positive for Las.
- (4) More than 100 citrus grove trees were tested by quantitative real-time PCR and conventional PCR at the Pico farm of the Horticultural Lab, USDA-ARS. The Las-infected citrus groves were used to evaluate the efficacy of screened chemicals in controlling HLB in the field. The experimental design was a randomized block design with three replications. The treatments were three rates of 1g/tree, 5g/tree and 10g/tree in combination with three the different injection methods; Direct-Inject QC (ArborSystems), Smart-Shot Injector (Treelogic) and Syringe-Injector. Citrus leaf samples will be collected every month for one year, and tested for Las by PCR, PS phytotoxicity and residues.