

### January 2010 progress report 3

Transmission of a pure culture of 'Candidatus Liberibacter asiaticus' (Las) by citrus psyllids into susceptible sweet orange plants is the initial step in completing Koch's Postulates. This project uses two mechanisms to introduce Las into the psyllids, membrane feeding and microinjection. We have done a number of experiments where Asian citrus psyllids were fed on sachets containing media with pure culture Las. The success rate of these experiments has been very low, but we report that we have successfully transmitted pure culture Las into sweet orange using psyllids. The infected trees are positive by real-time PCR, three months after inoculation, but do not show significant symptom development at this point. We are currently working to confirm the presence of Las in the inoculated trees using multiple methods including sequencing, but are reasonably confident that this represents the first success in the alternative confirmation of Koch's postulates via transmission by vector. In addition, we have begun conducting microinjections, beginning with control experiments where healthy psyllids were injected with hemocoel from Las infected psyllids. The microinjections were successful at multiple levels, as the injected psyllids survived, tested positive for Las, and were able to transmit the Las to healthy sweet orange seedlings. We plan to move forward with micro-injection of pure cultured Las, but at this point we have great difficulty obtaining adequate pure cultures and we have begun efforts to grow our own live, pure cultures of Las using various techniques.