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7.1 Acquisition of *Candidatus* Liberibacter asiaticus by the Asian citrus psyllid, *Diaphorina citri,* and the potential use of insecticides to prevent pathogen transmission.

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The spread of citrus greening disease or huanglongbing (HLB) in Florida is dependent on the transmission of the associated pathogen, *Candidatus* Liberibacter asiaticus, by the Asian citrus psyllid, *Diaphorina citri* Kuwayama. Several elements contribute to the spread of the HLB pathogen by *D. citri*, including acquisition period, latency of the pathogen in the psyllid prior to transmission, transovarial transmission, transmission efficiency, and vector competence. There are vast differences reported in the literature regarding these components of the vector-pathogen interaction. For example, in past studies with *D. citri* and the African citrus psyllid, *Trioza erytreae*, acquisition of the HLB pathogen is reported to require a minimum of 15 minutes to 24 hours and the psyllid feeding time for pathogen transmission to healthy plants from 15 minutes to 7 hours (Capoor et al 1974, Buitendag and von Broembsen 1993). In these and other past studies however, successful pathogen acquisition and transmission was determined by moving psyllids from HLB infected citrus plants to uninfected plants and subsequently monitoring the latter for symptom development. The vast differences in the results of these past studies likely are due to difficulties in separating symptoms caused by the HLB pathogen from non-pathogen related plant stresses (e.g. nutritional deficiencies). To date, a clear understanding of the vector-pathogen-plant interaction has not been established firmly using molecular techniques that now are available to researchers.

Use of insecticides has been promoted as one component of a successful HLB management program (da Graca 1991, Halbert and Manjunath 2004); however, few studies have attempted to directly measure the success of vector control with disease incidence. In a two-year study, Huang et al (1990) found that in adjacent citrus blocks, trees on a psyllid control program remained HLB free (based on visual symptoms) whereas those trees not receiving insecticide applications showed visual symptoms during the same period. While reports of apparently successful chemical control of psyllids for managing HLB are available from several countries (Chao et al 1979, Buitendag 1991), no studies have directly tested the ability of insecticides to prevent a treated plant from becoming infected when exposed to pathogen carrying psyllids. Given the reported lengthy duration of feeding time required for transmission to occur and the immediacy with which some insecticides cause mortality, detailed studies on the effects of insecticides on pathogen transmission may be useful in developing more effective psyllid control strategies to aid in the management of HLB.

As part of our larger study on this vector-pathogen interaction, here we present findings of two laboratory studies examining the rate of pathogen acquisition by adult and nymphal psyllids and the potential ability of soil-applied insecticides to prevent pathogen transmission via mortality of adult psyllids. To determine the rate of pathogen acquisition by adult *D. citri*, groups of 50-100 adult *D. citri* were confined on branches of potted citrus trees which had previously tested positive for the presence of the HLB pathogen using real-time PCR. Over a period of 1 day to 52 days, psyllid adults were removed from the plants and tested singly for the presence of the HLB pathogen using real-time PCR. Pathogen acquisition rate by psyllid nymphs was examined by caging single female psyllids on new leaf growth of HLB+ citrus plants for oviposition. After 7 days, the adult female psyllids were removed while the young leaves containing psyllid eggs remained caged until adult psyllids emerged. Those adults then were analyzed singly for the presence of the HLB pathogen using real-time PCR. The results of these caging studies showed that the HLB pathogen was detectable in 20-30% of psyllids which fed on plants as adults only, whereas up to 100% of the psyllid adults that developed from nymphs on HLB+ plant material tested positive for the presence of the HLB pathogen. These results, together with similar results from caging studies under field conditions, demonstrate that psyllids that complete their development on HLB+ citrus trees are more likely to acquire (and potentially transmit) the HLB pathogen than psyllids that fed on HLB infected trees as adults only.

The ability of systemic insecticide applications to prevent pathogen transmission also was examined. One hundred pathogen-free citrus seedlings were treated with either a soil-drench of imidacloprid or a water only control. Fourteen days after treatments were applied, ten adult psyllids from a colony reared on HLB+ citrus plants were caged on each seedling. After a confinement period of 72 hours, psyllids from each plant were removed, psyllid mortality assessed, and the presence of the HLB pathogen determined for each psyllid using real-time PCR. Plants were then held in the greenhouse for a period of 3 months after which they were assayed monthly using real-time PCR for the presence of the HLB pathogen. To better understand how insecticide applications may prevent pathogen transmission through disruption of feeding, an electrical penetration graph (EPG) was used to directly measure psyllid feeding behaviors on insecticide treated and untreated citrus plants and correlate those feeding durations with successful pathogen transmission. Results-to-date from these experiments will be presented and implications for development of psyllid management programs discussed.

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