

## **2.7 Is it possible to replant young groves in an area with endemic HLB – a hierarchical sampling approach to determine infection?**

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The acreage of Florida citrus has been declining and currently is the lowest since record keeping began in 1966. Among the reasons for the decline over the last couple of years is the lack of availability of nursery trees to reset existing groves and to plant or replant new groves. The lack of trees is due in part to the destruction of trees exposed or infected with citrus canker in 2005 and 2006 and was mandated by the citrus canker eradication program that was in effect until 2006. In addition, new regulations were put into place after the discovery of Huanglongbing (HLB) which resulted in a further reduction of available nursery trees while the nurseries built new enclosure structures that were compliant with the new pest and pathogen regulations.

After the discovery of HLB in Florida, many groves began to implement control measures that included aggressive psyllid control programs and the scouting and removal of infected trees. Despite the implementation of these management practices, many groves, especially in the southern portion of the state, are experiencing increasing infection levels. Given the current high level of inoculum pressure in these areas, it is unclear whether the increasing infection levels are a result of new infections or a result of pre-existing infections that are beginning to manifest HLB symptoms after a long latent period. In part due to the shortage of disease-free nursery trees and in part to the uncertainty of growers as to whether they can keep young trees free from HLB, some growers have chosen not to reset in existing groves and not to replant new groves until management practices can be developed that will allow newly planted trees to reach maturity with relatively low levels of HLB infection. Thus rate of infection in newly planted groves that are aggressively managed is an important factor to determine as growers decide whether they will replant and reset groves as trees are removed due to HLB. In this study, two relatively large, aggressively-managed commercial young-tree plantings are being followed to determine infection rates over time.

Two plantings, one 46 ha (115ac) in size and the other 54 ha (134ac) in size, were established in mid 2006. Together, both plantings total approximately 30,500 trees. Both plantings are located in counties with endemic HLB infection and in groves that had known previous HLB infections. One planting was located in a county that has moderate to high inoculum pressure and the other is located in a county with relatively low inoculum pressure. Both are being managed with what is considered to be an aggressive HLB management program by Florida standards. Although the programs differ slightly at each planting, the basic program consists of six inspections per year and 6-8 insecticide applications per year. In addition to the visual surveys, beginning in 2008, laboratory testing using real-time polymerase chain reaction assays (RT-PCR) was incorporated into the survey process to complement the field surveys based on visual symptoms.

RT-PCR testing is laborious, expensive, and requires specific expertise; therefore the amount of testing that can be performed is limited. However, RT-PCR is sensitive enough that samples can be bulked to some degree, thereby allowing more trees to be tested. Thus a survey method was designed that takes advantage of the sensitivity of the method and maximizes the amount of trees that can be sampled within given time and cost constraints. The survey method adopted for the RT-PCR based survey is a form of group testing that utilizes relationships between disease incidence at two levels in a spatial hierarchy, known as hierarchical sampling.

The first hierarchical sampling/RT-PCR testing of both groves was conducted beginning in August, 2008. At that time, none of the trees were showing visual symptoms. However, RT-PCR testing detected HLB in both of the young plantings. Based on the hierarchical sampling model, an estimated infection incidence of 0.44% and 0.37% was found in the plantings in the high inoculum and low inoculum pressure areas, respectively. Going forward, RT-PCR will be done on an annual basis and will be compared with infection incidence based on visual surveys.