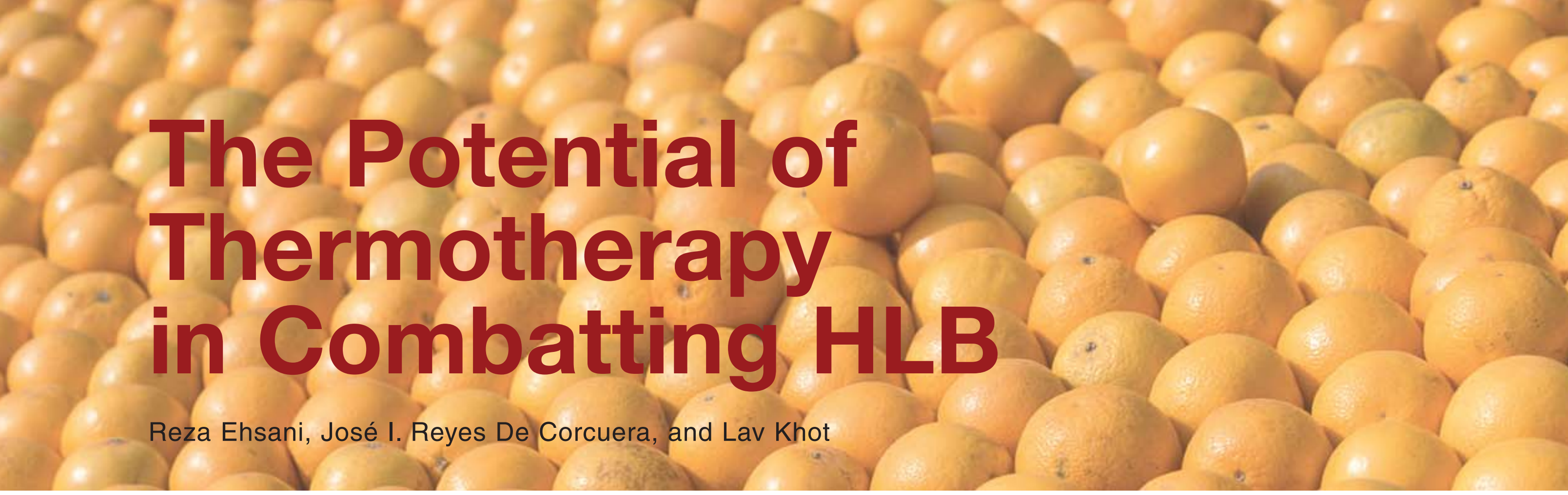

The Potential of Thermotherapy in Combatting HLB

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Florida has more than 182,109 ha (450,000 acres) of citrus orchards, with an estimated revenue of $8.9 US billion. However, since 2005, Florida citrus production has been seriously affected by Huanglongbing (HLB) disease, also called citrus greening. Citrus greening has cost the Florida citrus industry about $3.63 billion in lost revenue, as well as thousands of jobs. This devastating vector-transmitted disease is caused by a bacterial pathogen, *Candidatus* Liberibacter asiaticus (CLas), and spread by the Asian citrus psyllid (*Diaphorina citri* Kuwayama). This phloem-limited bacterium has spread to all citrus-producing counties in Florida. Infected orchards have a decline in yield of 10% or more due to fruit drop and reduced juice quality. At six months to about two years after infection, the trees develop symptoms such as chlorosis with a blotchy mottled pattern that results from starch accumulation. In most cases, under a regular orchard management program, infection results in tree decline, twig dieback, and death of the tree within four years. Moreover, during this period, the infected trees can serve as a source of inoculum, resulting in further spread of the disease.

So far, there is no known treatment for HLB, but citrus growers and stakeholders are making maximum efforts through research and development to find a way to control or treat this disease. Current management practices involve inoculum reduction through early disease detection and tree removal, pesticide and nutritional supplement applications in orchards with high levels of infection, and planting new trees. HLB management costs citrus growers an additional $736 per 0.4 ha (1 acre). In addition, environmental concerns regarding high pesticide use limit the feasibility of such approaches and have potential impacts on citrus byproducts derived from the peel. Moreover, ongoing research on genetically improved citrus cultivars resistant to HLB has not reached a stage where it can be applied for in-field control of HLB infection and spread. Therefore, there is an urgent need to develop novel technologies that can extend the productive life of infected trees until a more permanent solution is available.

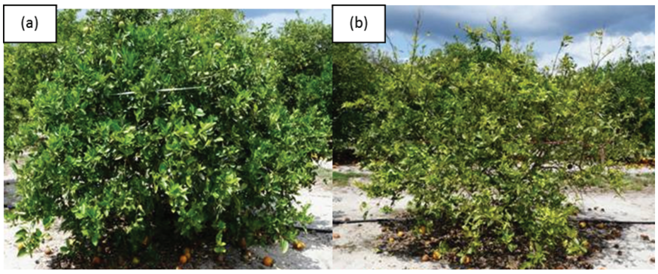
The challenges of thermotherapy

Thermal treatment of trees, or thermotherapy, has been used for decades in small potted trees to kill microorganisms and insects. A recent study published in *Phytopathology* showed that thermotherapy in a controlled greenhouse environment reduced HLB bacteria in seedlings and promoted healthy growth. Researchers and growers have taken this concept to the field on a small scale. By covering single trees with translucent plastic, tree canopies can be exposed to elevated temperatures. Thermal treatment of citrus trees in a grove is challenging because:

- The critical temperature and time required to kill HLB bacteria in a tree under field conditions is unknown.
- Adoption by the industry will depend on the ability to treat many trees in a short period of time.
- Higher temperatures that occur at the top of enclosed structures can injure the top of the canopy.
- The root system escapes the imposed heat treatment and will provide inoculum to re-infect the canopy.

Our team at the University of Florida's Citrus Research and Education Center (CREC), USDA scientists in Fort Pierce, Fla., and other scientists around the world are exploring thermotherapy to mitigate the effect of HLB on citrus trees. A key question that our research team is addressing is whether the level of HLB bacteria can be reduced to sustain the productivity of citrus trees in the short term until the ultimate solution is found.

An HLB infected tree with heat treatment enclosure.

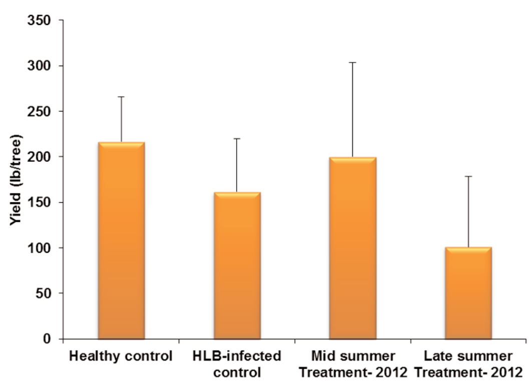


Symptomatic mid-summer treated (a) and untreated (b) citrus canopies nine months after thermotherapy.

Addressing the unknown

The exact mechanism by which heat treatment kills the HLB bacteria is not known known. The main mechanism of microbial inactivation is metabolic disruption, in particular protein denaturation. The activation of a virus inside the bacteria has also been hypothesized. To apply thermotherapy in the field, it is critical to know the temperature and duration of exposure required to significantly reduce the microbial population with minimal impact on fruit yield.

In 2012, we developed a prototype of a mobile system that uses solar radiation to heat treat HLB-infected trees in an orange grove. Within a plot of diseased trees, we covered only the trees that showed the most prominent HLB symptoms. Heat treatment is most effectively done from May to September, when there is enough solar radiation to bring the canopy temperature to an ideal level. During these months, orange trees are acclimated to hot weather and can better tolerate thermotherapy. At the CREC groves in Lake Alfred, Fla., we selected Valencia trees approximately 2.1 m (7 ft) tall that had visual HLB symptoms. The trees were covered with translucent plastic, one tree per day, for a five-hour heating period. In July 2012, the temperature inside the enclosure reached 45°C (113°F) or higher for at least four hours. Temperatures of 50°C (122°F) or higher were recorded at the top of the enclosure.



Fruit yield for thermally treated and control Valencia orange trees.

In May 2012, before the thermal treatment, the trees with HLB symptoms looked very similar to each other. Nearly a year later, in April 2013, the treated trees looked healthier than the surrounding untreated trees. Because of more cloud cover and cooler weather in late summer (September 2012), temperatures inside the enclosures did not reach 45°C (113°F) for as long as in mid-summer. The trees treated in late summer did not appear to be as healthy as the trees treated in mid-summer, but they appeared healthier than infected untreated trees, suggesting that thermotherapy was effective in mitigating HLB symptoms.

Data from the April 2013 harvest showed that fruit yield and juice quality from the mid-summer treated trees were not different from that of apparently healthy control trees. The mid-summer treated trees also had higher yields than the trees treated in late summer. Healthy control trees had the same mean soluble solids content (SSC) of 12.0° Brix as the trees that were treated in mid-summer. In contrast, untreated HLB symptomatic and trees treated in late summer had a mean SSC of 11.3° and 11.0° Brix, respectively.

Time and temperature matter

Thermotherapy using a plastic tarp was relatively slow and weather dependent. Although healthier trees resulted from thermal treatment above 45°C (113°F) for four hours, two or three hours were needed to reach that temperature even under the most favorable conditions, particularly in the interior and lower sections of the enclosure. In one instance, when ambient temperatures were low and conditions were windy, the temperatures inside the enclosure varied by 13°C (55°F).

Control over the rate of heating in the enclosure and a method to achieve uniform temperature distribution are needed. A cloudy or rainy day in summer could significantly reduce the temperature, thereby changing the effectiveness of the thermotherapy treatment. In our ongoing experiments, we are developing a rapid heating and circulation system to deliver uniform heat treatment to trees in an enclosure.

Optimizing the temperature and time to treat trees efficiently remains the next challenge. Would a tree survive if the temperature was raised but the time needed to reduce the bacterial population was reduced to minutes or seconds? That is the type of question that we will address with the second year of a project funded by a grant from the Citrus Research and Development Foundation. Our future work will focus on critical time-temperature relationships and automating the process of rapidly applying thermotherapy to orchard trees.

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