Controlling HLB with thermotherapy: What have we learned so far?

By Reza Ehsani, Megan Dewdney and Evan Johnson

In the last two years, many growers looked at thermotherapy as a way of keeping HLB-affected citrus trees in production until a more permanent solution could be developed. It has been shown that thermotherapy can kill HLB-causing bacteria under controlled environments. But the exact time and temperature is not known, and it is very difficult to determine since the bacteria cannot be cultured, and the current technique for determining live vs. dead bacteria has been very difficult to use.

There are many questions that still exist on the effectiveness of thermotherapy. Questions regarding thermotherapy can be divided into two groups: the engineering aspects and the science of thermotherapy treatment.

ENGINEERING ASPECTS

The engineering aspects of thermotherapy treatment deal with building systems and machinery that can effectively and economically heat-treat a large number of trees in a timely and efficient manner. All the engineering issues such as uniformity of heat distribution, efficiency, cost and productivity of operators and machines are considered under this topic.

As in many other mechanical systems, although there is always room for improvement, the existing machine satisfies these objectives. In the last two years, many versions of a thermotherapy machine using steam or vaporized hot water have been developed and are even being offered to growers as a service.

SCIENCE OF THERMOTHERAPY

The science of thermotherapy is trying to answers questions about how thermotherapy works, such as: Does the heat really kill the bacteria? If so, what percent of them are killed at a given dosage? Does heat trigger a response in the tree that alters the disease expression of HLB? What factors affect the repeatability and efficiency of thermotherapy treatment? What is the best time of the year to apply thermotherapy? What is the best duration and temperature combination to kill the bacteria while minimizing tree damage?

Unlike the engineering aspects of thermotherapy, the research on the science of heat treatment is lagging for two main reasons. One reason is that currently it is not possible to culture the HLB-causing bacteria (*Candidatus* Liberibacter asiaticus or *C*Las for short). So it is very challenging to determine the exact time and temperature requirement for killing *C*Las. To further complicate matters, there is not a reliable technique to quantify the live vs. dead *C*Las to get an accurate titer reduction estimate. Therefore, plant physiological health indicators have been used to measure changes in plant health and the potential effectiveness of the treatments. These physiological indicators include plant water uptake rate, leaf area index, normalized difference vegetation index (NDVI), fruit set, yield and fruit quality.

FIELD TRIAL UNDERWAY

A comprehensive, replicated field trial was established to determine the effect of thermotherapy using steam on *C*Las titer and reinfection (from roots), overall tree health, and fruit yield and quality. In this trial, trees were subjected to thermotherapy in each of the four seasons in the course of a year, with a three-month interval between treatments, to evaluate the most appropriate time for thermotherapy. Each trial had one control (no heat treatment) and five treatments: 131°F maintained for 0 seconds, 131°F maintained for 60 seconds, 131°F maintained for 90 seconds, 131°F maintained for 120 seconds, and finally, the extreme treatment of 140° F maintained for 30 seconds. Different sets of 300 trees were treated each time for different durations and temperatures to study the effect of the time-temperature interaction and the time of year on tree response and yield.

Before and after treatments, leaf and root samples were collected with continuous sampling occurring over time to determine the proportion of bacteria killed and the movement of bacteria from roots to the treated branches and leaves. Damage caused to the canopy by thermotherapy is being

quantified. Both the fresh weight of wood pruned so that the machine will fit over the trees and the dry weight of branches killed by the steam treatment were measured. Fruit drop resulting from thermal damage to fruit on the tree was also assessed, and multi-band aerial images were obtained every two months after treatment. Yield and fruit-quality assessment will be done in the Valencia harvest season for the next three years.

INITIAL OBSERVATIONS

The trial will be continuously monitored, and data will be collected for three more years to see the effects of long-term heat treatment. Although it is too early to make any conclusions, the following observations have been made so far:

The variability of *C*Las in the tree is very high. Therefore, a large number of leaf samples need to be collected to get an accurate picture of the viability of the bacteria before and after heat treatment. It was estimated that at least 36 leaf samples (5 leaves each) had to be collected per tree.

Figure 1 (page 28) shows the effect of different heat treatments on the percent change of NDVI value for trees treated at different times and temperatures. NDVI is a vegetation index that has been used as a measure of plant health. In this study, NDVI was measured from aerial images, and its average value was calculated for each tree. Aerial images were taken with an unmanned aerial system every two months after heat treatment.

NDVI values correlate with the vegetative growth of trees after heat treatment over time and are being used as an indicator of plant health. Negative values of NDVI in Figure 1 for each treatment show overall NDVI decline, and positive values show improvement in NDVI value after heat treatment. The treatment of 140° F for 30 seconds and 131° F for 120 seconds had the greatest effect on change in the value of NDVI in the two months after canopy heat treatment. The NDVI value decreased for the control and

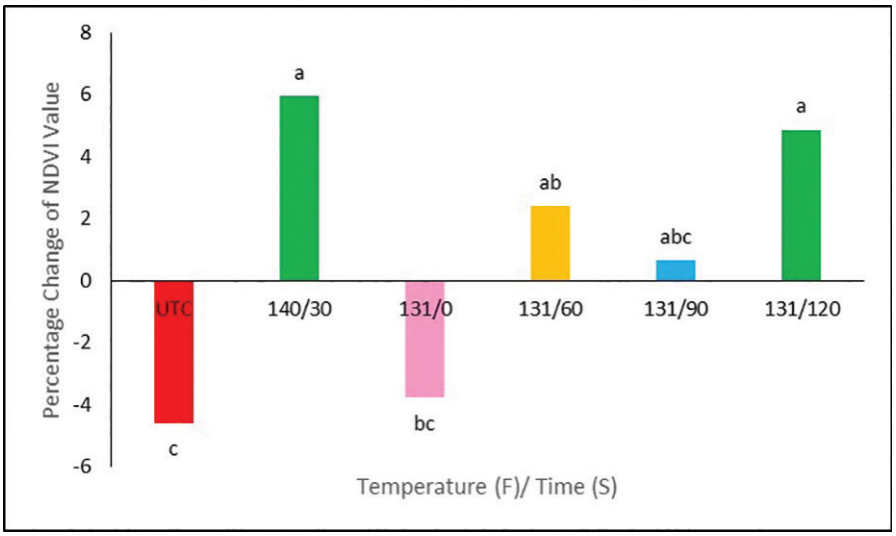

Figure 1: Percent change of NDVI value for different heat treatments after two months

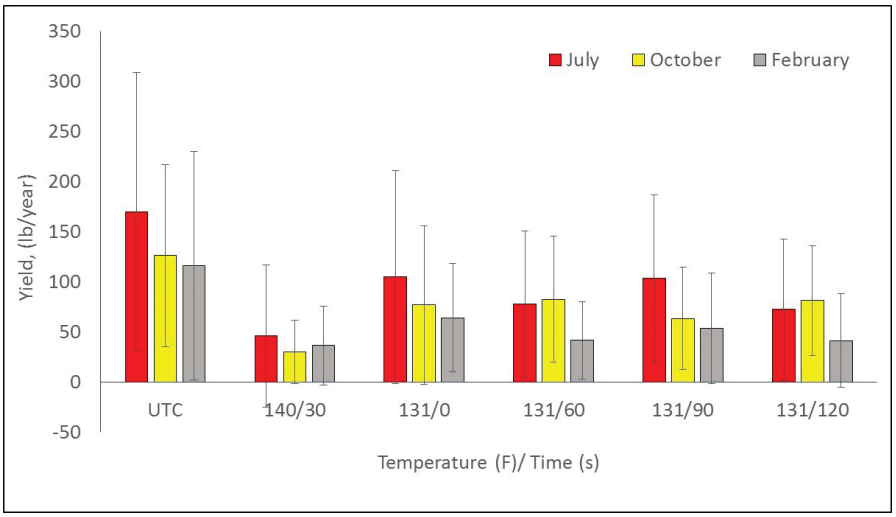

Figure 2: Effect of heat treatment on citrus fruit yield after the first year of thermotherapy treatment in three different months is shown.

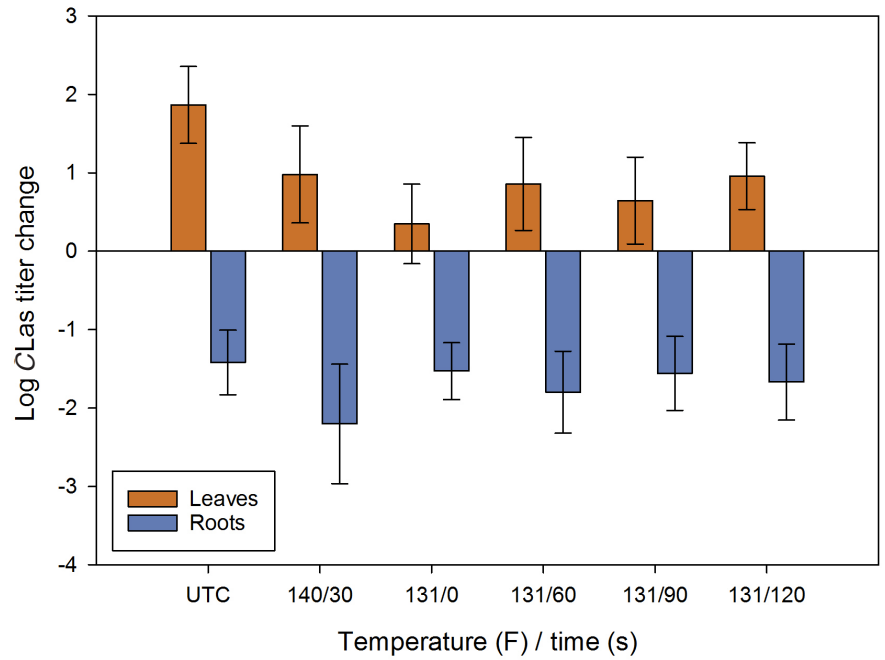

Figure 3: The effect of summer (July) thermotherapy treatment on *C*Las titer one month after treatment compared to pretreatment titers

short-duration, heat-treated trees.

Figure 2 shows the effect of heat treatment on the yield for the different treatments. Overall heat treatment had an adverse effect on yield in the first year, which can be attributed to heat-induced fruit drop. Yield reduction was greater in trees that were treated at a higher temperature.

In the summer treatments, the bacterial titer in the roots was reduced in all treatments including the untreated control. This observation suggests an upward migration of bacteria, since the roots were not exposed to heat (see Figure 3). In the untreated control trees, the reduction of root titer corresponded to an increase in the bacterial titer within tree canopies. However, the bacterial titer in the canopies of all the thermotherapy treatments did not increase as much as the untreated control, indicating there was a titer reduction due to the thermotherapy treatments. No treatment achieved a zero bacterial titer in the canopy.

The seasonal migration of *C*Las during the summer made it impossible to determine if the detection of bacteria was due to a failure to reach the *C*Las kill temperature or the rapid recolonization from the roots. The data from the remaining time periods have not been fully analyzed at this time.

In summary, observations from the first year indicate that heat treatment in the spring and summer is more effective based on general canopy health. Also, the bacteria from the roots can move to shoots faster than what was expected, and this may require multiple heat treatments.

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