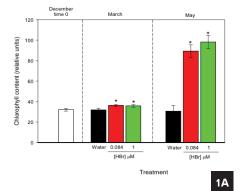
### Effects of homobrassinolides on HLB-affected trees in Florida

By Fernando Alferez, Christopher Vincent and Tripti Vashisth

n the current scenario of widespread infection and severe symptoms of HLB in Florida, horticultural practices that improve plant health in the presence of the disease are needed. For this reason, we sought to follow up on reports of positive effects of a brassinosteroid on infected plants. Brassinosteroids are a relatively newly discovered group of plant hormones that regulate multiple developmental and physiological processes. Most interestingly, brassinosteroids induce disease resistance in a great number of crop plants against bacteria, fungi, oomycetes and viruses.



Brassinosteroids are present in two active forms, epibrassinolide (EBL) and homobrassinolide (HBr).

Recently, EBL was shown to reduce *Candidatus* Liberibacter asiaticus (*CLas*) bacterial titer and alleviate symptoms of HLB in mature citrus trees in Cuba. The results of the Cuban study, over a 12-month period, were impressive: In a greenhouse trial, bacterial titers were reduced to non-detectable levels. In the field, a

seven-fold reduction of bacterial titer was measured.

This effect seems to be mediated by the activation of a large number of defense-related pathways in the tree. However, the long-term effects of this treatment on tree health and fruit yield and quality were not assessed.

HBr is commercially available in the United States and has been proven effective in increasing resistance to disease in other plants such as





**Figure 1.** Effects of HBr on tree phenology. 1A: Chlorophyll content in new leaves over time. 1B: New leaves after full expansion (representative leaves sampled in July). 1C: Blooming in HBr-treated [1 micrometer (1  $\mu$ M)] trees (right tree) as compared to non-treated trees (left tree) on February 20, 2018. 1D: HBr-treated tree canopy after summer flush (July).

tobacco and apple. Hence, there is an opportunity for testing HBr in citrus trees grown in Florida under HLBprevalent conditions.

A research project, funded by the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Citrus Research Initiative, started in December 2017 with the overall goal of assessing effects of HBr on citrus tree health, fruit yield and quality. The project also includes assessing CLas bacteria titer in the central and southern citrus-producing regions of Florida to ensure that results are applicable statewide. Researchers are conducting experiments in groves in Lake Alfred at the Citrus Research and Education Center and in Immokalee at the Southwest Florida Research and Education Center (SWFREC) using the same varieties (6- to 8-year-old Valencia trees on Swingle rootstock) and experimental design.

Starting in December, spray treatments were performed every two weeks for six months at both locations, following the dosage and frequency published in the Cuban study. Treatments were: 1) water (control), 2) 0.084 micrometer (µM) HBr and 3) 1 µM HBr. Fruit detachment force (FDF), fruit drop and fruit peel coloration were tested. Leaves were collected from every individual tree monthly to measure bacterial population. Timing of flushing and blooming in treated trees was also assessed. Canopy volume, trunk diameter and leaf chlorophyll content in new, fully expanded leaves were examined as well.

Although the experiment is still in its first year, we can report on certain trends in tree health, fruit yield and quality. The treated plants must be followed further before recommendations can be made to Florida growers.

#### **BACTERIAL POPULATION**

Bacterial population was assessed monthly by real-time polymerase chain reaction according to U.S. Department of Agriculture Animal and Plant Health Inspection Service recommendations. Prior to the start of the experiment, all trees were HLB positive. After six months, no significant differences were observed between control trees and trees that received HBr.

(Continued on page 20)



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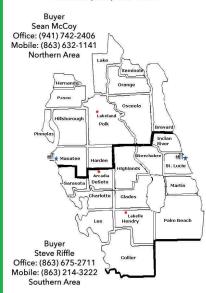
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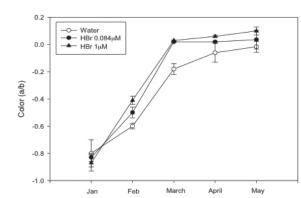




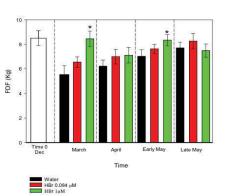
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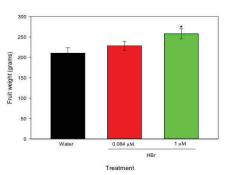


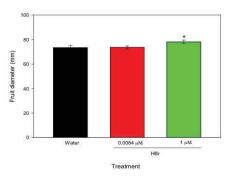
**Figure 3.** Fruit detachment force (FDF) in response to the different treatments. FDF was measured with a pull force electronic gauge. Thirty fruits per treatment were measured monthly.

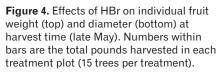
#### TREE PHENOLOGY

Chlorophyll content was evaluated non-destructively at two different times after the first application in December: 1) in March, after new leaves were fully expanded and 2) in May, when these same leaves were mature. Data show that chlorophyll content was significantly higher in leaves from both HBr treatments, and these differences increased with time (Figures 1A and 1B, page 18). There were no differences in canopy volume and trunk diameter among different treatments, though it is likely that six months is too early in the course of the study to find whole-plant differences.

Noticeably, we found that blooming occurred 10 days earlier in HBr-treated trees (irrespective of the dosage) as compared to the nontreated trees, and flowering was profuse and uniform (Figure 1C, page 18). These effects were more pronounced in trees at the SWFREC experimental site. Trees showed denser and darker-green canopies after the June flush Figure 2. Color (a/b index) development in flavedo during fruit maturation. Thirty fruits per treatment were marked at the beginning of the experiment. Color was measured along the equatorial zone of each fruit with a portable colorimeter.







when compared to the beginning of the experiment (Figures 1C and 1D, page 18).

#### FRUIT YIELD AND QUALITY

Color measurements were taken monthly using a portable Minolta color meter. Color break and development occurred earlier in trees treated with HBr at both concentrations compared with untreated trees (Figure 2). This effect disappeared over time and by the end of maturation (May 25, 2018). There were no significant differences in fruit coloration.

FDF was higher at higher HBr concentrations in March and early May (Figure 3). Total fruit yield at the end of the experiment was higher in trees treated with higher HBr concentration (242.5 pounds) compared with trees treated with the lower concentration (210.1 pounds) and with water controls (191.7 pounds). Average fruit weight and diameter increased significantly in response to the higher HBr concentration (Figure 4, page 20). The higher retention force of the fruit to the tree may explain the increase in yield at the end of the experiment.

Average fruit weight and diameter increased significantly in response to the higher HBr concentration.

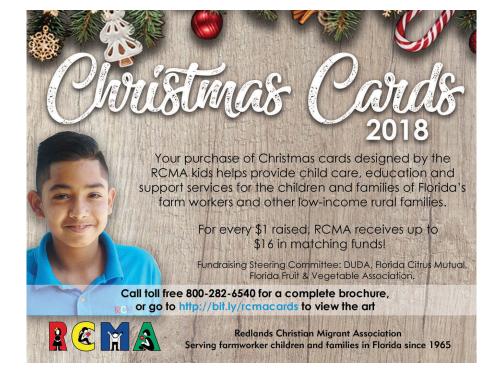
#### CONCLUSIONS

The effects observed after the treatments with HBr were not what we initially expected, as we were more interested in assessing reduction in bacterial infection in the citrus trees. However, we observed advanced blooming date and acceleration of fruit maturation. Trends regarding tree health, fruit yield and quality observed after HBr treatments are worth following.

Determining appropriate timing of product application may allow HBr to be used as a new management tool for Florida citrus growers. The lack of effect on bacterial population could be due to the fact that the study is still in its early stage. Nevertheless, results from our study suggest that HBr treatment may improve tree health and, therefore, tolerance and productivity of infected trees. The study will continue for an additional year to investigate whether homobrassinolide applications are a suitable management practice to combat HLB.

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Fernando Alferez, Christopher Vincent and Tripti Vashisth are UF/IFAS assistant professors.



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