Antibiotic delivery methods: Trunk injection vs. foliar application

By Nabil Killiny and Christopher Vincent



he citrus industry has suffered numerous setbacks over the past 20 years because of the devastating effects of huanglongbing (HLB) disease. Many options for controlling the disease are being investigated to make groves as productive as possible under the current HLB pressure. Even small improvements in tree health or yield of diseased trees may help growers survive until either a permanent cure is found or citrus trees with proven tolerance to the disease are widely available.

One of the approaches under investigation has been the use of antibiotics. University of Florida research has addressed the use of foliar applications of antibiotics and whether these can be improved with the use of commercial adjuvants.

Antibiotics have been successfully used to treat several diseases, including fire blight of apple and pear, leaf spot disease of peach and nectarine, and lethal yellowing disease in elm and coconut palm trees. The use of antibiotics was also proposed for the control of HLB in the 1970s after the disease was shown to be caused by a microbial plant pathogen. Early studies showed that trunk injection of tetracycline significantly reduces the HLB symptoms in infected citrus trees.

In the last few years, the use of antibiotics was again proposed for the control of HLB due to the significant damage caused by the disease in Florida. In 2016, the Environmental Protection Agency approved the foliar application of oxytetracycline and streptomycin for the treatment of HLB in Florida.

OXYTETRACYCLINE UPTAKE

Recently, we conducted a field study to evaluate the efficiency of foliar application of oxytetracycline and to compare it with trunk injection. We studied the effect of penetrant adjuvants on the uptake of oxytetracycline upon foliar application. In this study, we tested nine different adjuvants and compared them to trunk injection. Our results showed that only a trace amount of oxytetracycline ($< 0.4 \mu g/g$) was taken up by citrus leaves upon foliar application.

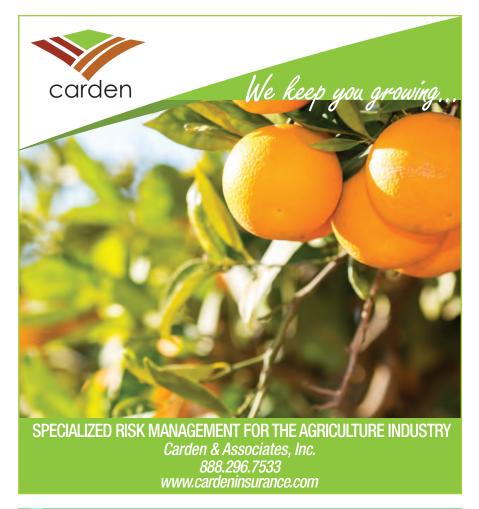
Similar results were also obtained in the greenhouse. Adjuvants did not increase the uptake of oxytetracycline by citrus leaves upon foliar application. On the other hand, a high level of oxytetracycline ($\sim 6~\mu g/g$) was detected in the leaves of the trunk-injected trees. The bacterium that causes HLB (*Candidatus* Liberibacter asiaticus, *CLas*) was significantly reduced in oxytetracycline-injected trees 30 days after treatment, whereas it was not affected by any of the foliar applications of oxytetracycline.

Study results showed poor uptake of oxytetracycline by intact citrus leaves, indicating low penetration of oxytetracycline through the citrus cuticle. Transmission electron microscopy for citrus leaves showed that the upper leaf surface was protected by a thick, waxy and compact cuticle that has no stomata.

On the other hand, perforating the citrus leaf cuticle with laser light improved the uptake of oxytetracycline and reduced the *C*Las titer in the field. An increase in the uptake of a fluorescent glucose was observed after perforating citrus leaves with the laser. These results suggest that the citrus cuticle is the main barrier preventing adequate uptake of oxytetracycline.

ANTIBIOTIC MOVEMENT

In an earlier study, we compared root drench and stem delivery of oxytetracycline and streptomycin in citrus seedlings. High levels of oxytetracycline and streptomycin were detected in the phloem, xylem and leaves with both methods. However, low levels of oxytetracycline and streptomycin were detected in the roots after stem delivery, indicating that the downward movement of

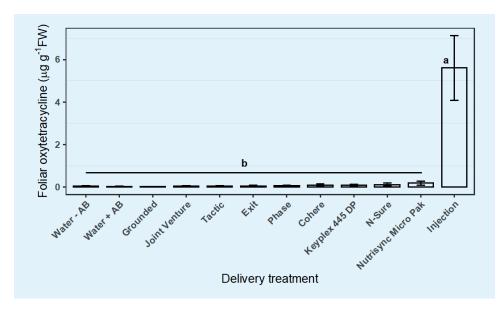






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Delivery of oxytetracycline (OTC) by injection or by foliar application with one of any of the listed agricultural adjuvants. "Water – AB" means water with no OTC. "Water + AB" means water with FireLine 17 WP. The OTC rate for all treatments other than "Water – AB" was 1.56 grams of OTC per tree.

these antibiotics was limited.

The presence of oxytetracycline and streptomycin at high levels in the xylem indicated that these antibiotics were taken up by the xylem and translocated to other tissues. To test this hypothesis, we applied oxytetracycline by root drench and trunk injection to girdled and non-girdled citrus trees. In both cases, oxytetracycline was

detected above the girdle, indicating that the xylem was the main path for oxytetracycline translocation, and movement into the phloem occurs after xylem translocation.

Although antibiotics have been successfully used for the control of several plant diseases for more than 70 years, their use has been challenged by several factors. Thus far, trunk injection is by far

the most effective method of delivery. Foliar methods deliver much less effectively and efficiently than injection.

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