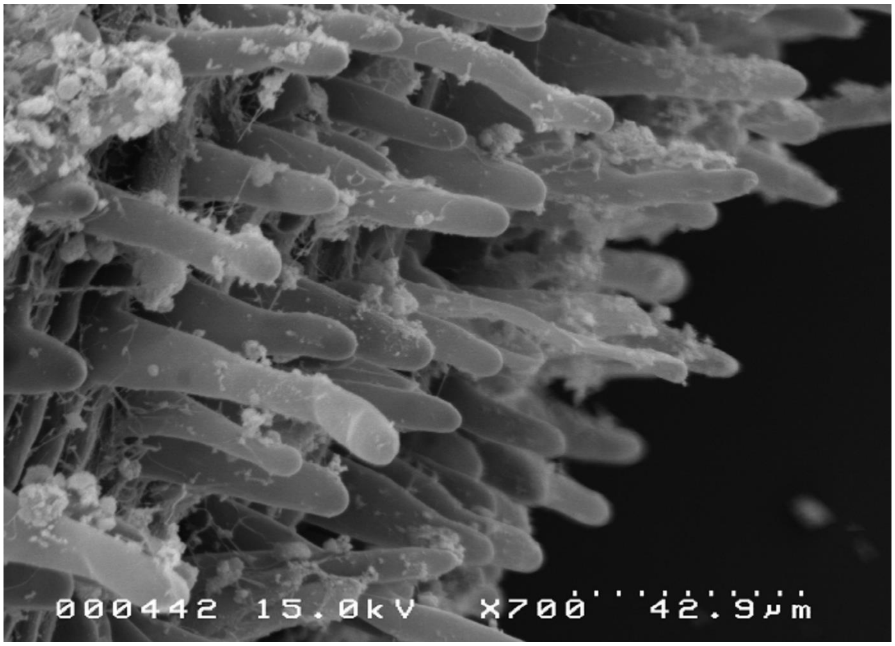

Figure 1. Root hairs were observed on Carrizo citrus fibrous roots in the presence of solid tricalcium phosphate and low-solution phosphorus (tank 2).

Root hair enhancement with low phosphorus concentrations

By Laura Waldo and Arnold Schumann

It has been the accepted belief for many years that citrus varieties and rootstocks do not develop root hairs. However, research has shown that root hair growth can be stimulated when soil phosphorus (P) concentrations are extremely low. It is possible that in Florida, citrus roots could fail to produce root hairs due to soil conditions with abundant available P.

WHY ROOT HAIRS ARE IMPORTANT

In the HLB-endemic Florida citrus-production regions, most citrus trees suffer from nutritional deficiencies as a result of a decrease in fibrous root mass. These fibrous roots are less than 1 millimeter in diameter and responsible for more than 75 percent of the nutrient uptake from the soil. Having an abundance of root hairs would greatly increase the surface area of the root system (up to 6x increases are reported in the literature), thereby increasing the ability of the plant to take up more nutrients and water.

Solid evidence for the uptake of most major nutrients and micronutrients by root hairs now exists. The primary reason for root hair existence is to increase the efficiency of nutrient ion uptake from the soil when concentrations are low. Phosphorus concentration seems to be pivotal for regulating root hair growth.

TESTING TRICALCIUM PHOSPHATE

Research has shown that root hairs could be induced in eight different plant species when available P concentrations were kept at low, but not deficient, concentrations in solution. When bioavailable P was kept at high or very low concentrations, root hairs failed to develop.

Tricalcium phosphate (TCP) is a sparingly soluble calcium salt of phosphoric acid and is also known as tribasic calcium phosphate and bone phosphate of lime. The solubility of TCP is pH-dependent; as pH decreases (increase in acidity) the release of P increases. Importantly, the process can be reversed, meaning that precipitation of solution P back onto the solid phase TCP can occur if the pH and solution calcium are increased. In theory, maintaining a soil pH in the 6.5 to 7.0 range can maintain an equilibrium of solution P in low concentrations, when TCP is the sole source of added P fertilizer.

To test this theory, Carrizo citrange seedlings that were germinated three months prior to this study were selected. Twenty-seven uniformly sized seedlings were placed into three solution culture tanks (9 seedlings each). The three different liquid culture hydroponic solutions were developed to maintain dissolved P concentrations. The concentrations were high (10 ppm), low (0.7 ppm) and very low (0.2 ppm).

Solutions were refreshed often to maintain nutrients in their prescribed concentrations. Tank 1 (control, high P) contained a complete nutrient solution with soluble potassium phosphate as the only source of P; pH was adjusted to 6.8 using sodium bicarbonate. Tank 2 (low P) contained an equivalent ratio of nutrients as the control fertilizer. However, solid TCP was used as the sole source of P in solution (pH approximately 6.8). Tank 3 (very low P) used the same nutrient solution plus TCP as tank 2, but calcium carbonate was added to buffer the solution pH above neutral (approximately 7.2).

The seedlings were maintained in solution culture for six months before they were sampled to examine root hair growth. Root sampling for quantification of root hairs was done using scanning electron microscopy. Several roots per tank were sampled, and images were taken at 300x magnification from each treatment for counting root hairs.

Two weeks before sampling roots, the solution cultures were tested for the release of phosphate-P from TCP. At pH 6.8, the TCP buffers the solution P at concentrations between 0.54 and 0.92 ppm (tank 2). At a slightly higher pH, 7.2, TCP buffers the solution P at concentrations between 0.05 and 0.49 ppm (tank 3). Although the concentration of P in solution was low, the leaf P concentration of the plants in tank 2 was 0.24 percent, which is above the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) recommended optimum nutrient threshold concentration of 0.12 to 0.16 percent. In tank 3 (very low P) however, leaf P concentrations were 3x lower (0.08 percent), which is below the critical deficiency threshold of 0.09 percent.

Tank 2 (low P) had a significantly higher amount of root hairs develop than did tank 1 (high P) and tank 3 (very low P). TCP at pH 6.8 (tank 2) induced a low-P environment that was beneficial for root hair development (Figure 1, page 22), with greater than 1,000 more root hairs per square millimeter than tank 1 (high P), and almost 2,000 more root hairs per square millimeter than tank 3 (very low P). These abundant root hairs are able to seek out and scavenge nutrients (not only P) in solution with their greatly increased surface area in order to keep plants healthy.

PSYLLID EXPOSURE

After the initial quantification of root hairs took place, the tanks were placed into insect cages and exposed to wild-caught psyllids for one month in order to inoculate the trees with *Candidatus* Liberibacter asiaticus (*C*Las), the reported causal agent of HLB. Two months after exposure to psyllids, the leaves were sampled and tested with qPCR for the presence of *C*Las. Roots were sampled again for quantifying root hairs, and leaves were sampled for nutrient status.

After exposure to the psyllids, all the trees tested positive for HLB, with qPCR cycle threshold (Ct) values ranging from 30.0 to 36.5 (Figure 2, page 24). According to these results, the tank 2 (low P) treatment trees had the lowest *C*Las titer (Ct = 36.5), and the high-P (tank 1) treatment trees had the highest *C*Las titer (Ct = 30). Although HLB disease reduced root hair growth

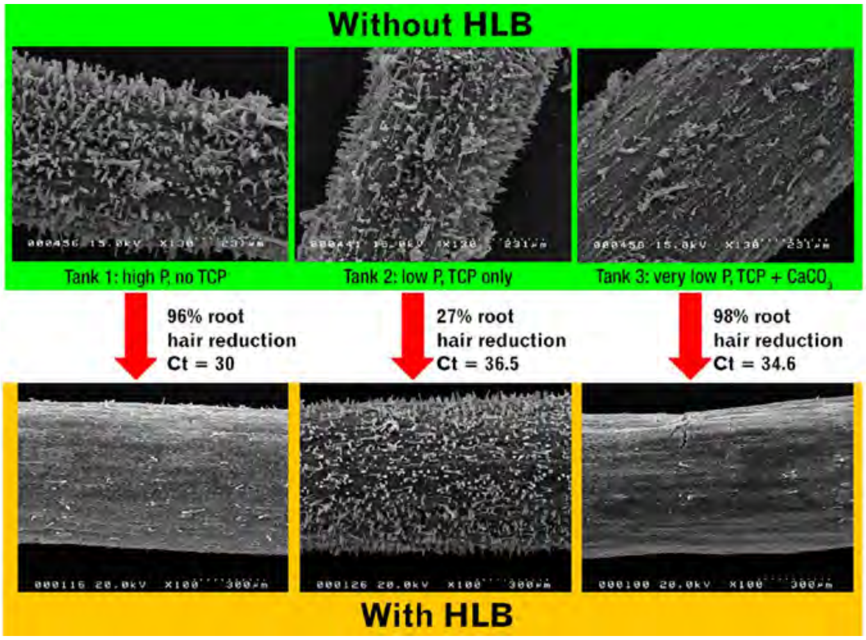

Figure 2. Scanning electron microscopy images of Carrizo citrus roots were taken prior to and after HLB infection. The text next to the red arrows indicates changes in root hair density after HLB inoculation and final qPCR status.

in all treatments, the low-P (tank 2) treatment maintained the highest density of root hairs (27 percent reduction) after being inoculated with *C*Las. In contrast, the high-P and very low-P treatments resulted in a nearly complete collapse of root hair growth after *C*Las inoculation (96 and 98 percent root hair reduction, respectively).

Although additional research is required, using TCP to maintain solution P at low concentrations may be a novel approach for increasing root hair proliferation in HLB-endemic citrus groves, potentially improving nutrient uptake and increasing citrus tree health. Mycorrhizal associations with roots can also enhance their surface area for nutrient uptake, with benefits that are like root hairs. A low-P environment is most conducive for optimal mycorrhizal performance.

Acknowledgements: The funding for this research was provided by the Citrus Research and Development Foundation. The authors thank Daniel Stanton and the Citrus Research and Education Center (CREC) microscopy laboratory for help with the microscopy, Evan Johnson's laboratory for the qPCR analyses, and Napoleon Mariner, Timothy Ebert and William Holmes for designing and maintaining the hydroponics experiment.

Laura Waldo is a senior biological scientist and Arnold Schumann is a professor, both at the UF/IFAS CREC.