Buddha's Hand citron could play role in canker resistance breeding

By Naveen Kumar, R.C. Ebel and P.D. Roberts

Citrus canker became endemic in Florida after several introductions and eradication programs dating back to 1915. The citrus industry struggles for an effective, permanent program. Canker is an expensive disease due to the need to increase the number of sprays and products applied. Canker also causes enormous economic losses due to fruit drop and reduction in export quality.

Generally, citrus growers spray copper compounds to control this disease. Copper is a trace element, micronutrient and potential environmental pollutant. In due course, bacteria may develop resistance against this metallic pesticide.

NATURAL RESISTANCE

To identify other canker management options in citrus groves, the Southwest Florida Research and Education Center (SWFREC) lab was actively engaged in identifying natural resistance to citrus canker. An article from Citrus Industry in February 2013 reported the mechanisms of natural canker (*Xanthomonas citri* subsp. *citri; Xcc*) resistance in kumquat. However, certain physiological limitations prevent its use in current breeding programs. Kumquat presents an avoidance mechanism (leaf defoliation) for canker resistance. Canker-infected leaves defoliate after artificial inoculation within one week, curtailing photosynthate availability to developing fruits and concomitant decline in fruit yield. In addition, there are reports that showed kumquat also exhibits typical canker symptoms when artificially inoculated with the pathogen and planted with sweet orange in groves.

As another research project, we further evaluated the sources of natural resistance in Buddha's Hand citron (*Citrus medica* var. *sarcodactylis*). This small ornamental tree is characterized by the human finger-like appearance of mature fruit. The rind of the fruit is highly fragrant, and fruit size varies from 7 to 12 inches. Fruit lack pulp and are dominated by thick albedo. In China, Buddha's Hand is popularly known as Fo Shou and is used as a symbol of long life and happiness.

AFTER ARTIFICIAL INOCULATION

Earlier disease development of canker in Buddha's Hand is similar to other genotypes following artificial inoculation of *Xcc.* Characteristics include water soaking and raised epidermis, which were evident at four and six days after inoculation (DAI) (Figure 1). However, the most striking and conserved features were

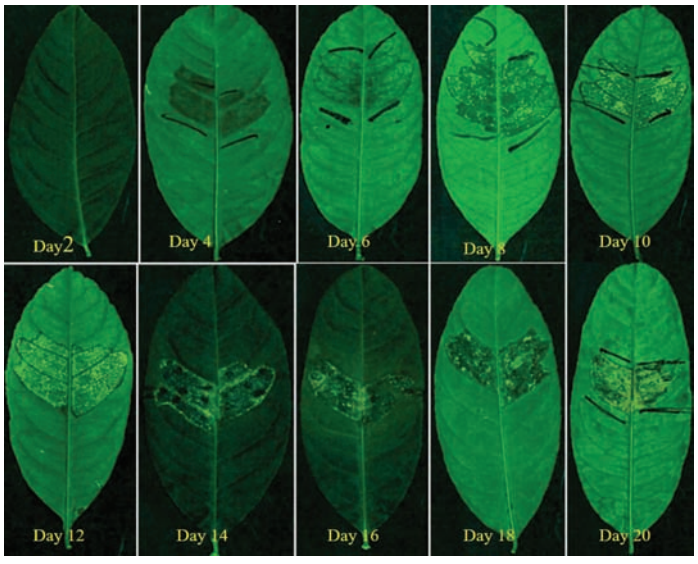

Figure 1. Visual symptoms of canker development in Buddha's Hand. The number at the bottom represents the number of days after inoculation (DAI).

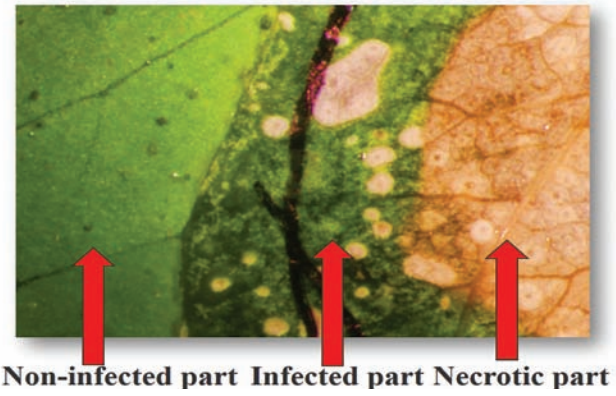

Figure 2. Microscopic view of the epidermis of an *Xcc*-infected leaf of Buddha's Hand.

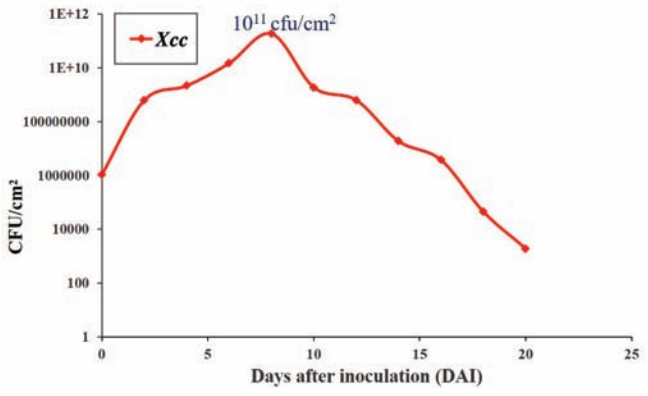

Figure 3. Bacterial population (colony forming units/cm^2) in Buddha's Hand leaves.

observed at eight DAI: 1) the development of small necrotic zones that later transformed into large necrotic areas (figures 1 and 2, page 20), 2) the epidermis remained intact, which is notably ruptured in other citrus genotypes (Figure 2, page 20) and 3) infected leaves were retained on the infected plant between 20 to 30 DAI.

These characteristics are of ecological significance because: 1) formation of a necrotic zone acts as a barrier restricting the movement of *Xcc* to non-infected parts of the leaf (figures 1 and 2, page 20), 2) an intact epidermis prevents the spread of inoculum through wind-driven rain and wounds on citrus and 3) delayed abscission means that leaves are still supporting the photosynthates supply to fruits. Buddha's Hand is unique from other commercial varieties of citrus because it manifests these positive responses.

Following artificial inoculation, *Xcc* populations transiently increased and then declined by a factor of 10^8 in Buddha's Hand leaves at 8 DAI (Figure 3, page 20). Data showed active resistance against citrus canker. Such a drastic decline in *Xcc* populations was not observed in kumquat. Kumquat only showed a 10^3 factor decline in *Xcc* populations, which is followed by excessive defoliation.

BIOCHEMICAL BASIS

We also studied the biochemical basis behind this significant decline in *Xcc* populations in terms of hydrogen peroxide production and lipid peroxidation of membranes. Both of these indices have been shown to cause adverse effects on the host, as well as on pathogen populations. In our work, a higher concentration of hydrogen peroxide and lipid peroxidation coincided with active decline in *Xcc* populations. Several reports have shown that lipid peroxidation of membranes generate intermediate metabolites that act as a precursor for defense hormones like jasmonic acid. Jasmonic acid is a defense hormone and can initiate cell death program.

These active canker resistance responses identified in Buddha's Hand are just the first steps in understanding the full potential and mechanisms of canker resistance for eventual use in canker resistance breeding programs.

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