Tristeza

P.D. Roberts, R.J. McGovern, R.F. Lee, and C.L. Niblett, Respectively, Assistant Professor, Southwest Florida Research and Education Center, Immokalee, Fl 34120; Associate Professor, Gulf Coast Research and Education Center, Bradenton, Fl 34203; Professor, Citrus REC- Lake Alfred, Fl 33850 and Professor Plant Pathology Department, University of Florida, Gainesville, Fl 32611. Revised April 2001

Florida Cooperative Extension Service / Institute of Food and Agricultural Sciences / University of Florida / Christine Waddill, Dean

Tristeza, caused by the citrus tristeza virus (CTV), is one of the most important factors limiting citrus production worldwide. The virus originated in Asia and has been spread by man to most citrus producing areas. Devastating epidemics have occurred in the western hemisphere beginning with Brazil and Argentina in the 1930s, when both severe CTV strains and the efficient aphid vector, *Toxoptera citricida*, were introduced by importation of living citrus material. Tristeza was first reported in Florida in the early 1950s, but serious losses were not experienced until the 1980s after CTV strains causing decline (CTV-D) on sour orange rootstock had become widespread. The epidemic of CTV-D on sour orange rootstock in Florida increased with the arrival of *T. citricida* in 1995 and has effectively eliminated sour orange as a rootstock for Florida citrus.

It is essential that steps be taken to prevent the introduction and spread of more damaging CTV strains such as those that cause stem-pitting of grapefruit, lime, and sweet orange scions regardless of the rootstock. The presence of such strains could severely limit production of grapefruit, lime and vigorous sweet orange varieties such as Hamlin and pineapple.

Causal Agent and Epidemiology

Citrus tristeza virus, a member of the closterovirus group, is the largest known plant virus, having the shape of a long flexuous rod about 11 X 2,000 nm in size (Figure 1). The genetic material of the virus consists of a single strand of ribonucleic acid (RNA) which is enclosed by a protein coat. Virus spread occurs through use of CTV-infected budwood and by aphids. CTV is not transmitted in seed, and it is unlikely that it moves in the field on contaminated tools and equipment.

CTV is transmitted by several aphid species in a semi-persistent manner. Aphids may acquire and transmit the virus during short feeding probes, but longer feeding times (up to 24 hours) increase the efficiency of virus transmission. Viruliferous aphids are capable of transmitting CTV for 24 hours after its acquisition. Four aphid vectors of CTV are present in Florida: 1) the melon aphid, *Aphis gossypii*, which is yellowish-green to blackish-green; 2) the spirea aphid, *A. spiraecola*, which is light green; 3) the black citrus aphid, *Toxoptera aurantii*, which is shiny black; and 4) *Toxoptera citricida*, commonly called the brown citrus aphid, which is also shiny black. *T. citricida* can an be differentiated from *T. aurantii* by antennae color and wing veination using a 20x hand lens (Figure 2). *T. citricida* was first found in Florida in 1995 and has spread throughout the state. *T. citricida* is the most efficient aphid vector of CTV, about 20 times more effective in virus transmission than *A. gossypii*, the next most efficient vector. Even more alarming is the demonstrated ability of *T. citricida* to transmit severe stem pitting CTV strains that are not readily transmissible by the other three aphid vectors. In addition, *T. citricida* reproduces very rapidly and reaches much higher populations than other vectors of CTV.

The introduction of stem-pitting CTV strains now, in the presence of *T. citricida*, could have the same disastrous impact on Florida citrus production that occurred in several South American countries.

Host Range and Symptoms

CTV infects almost all citrus species, hybrids, and relatives. Many strains of the virus exist and contribute to the diversity of symptoms associated with CTV infection. Mild strains of CTV produce essentially no noticeable symptoms in their hosts. Severe strains produce a range of symptoms including one or more of the following: seedling yellows; decline on sour orange rootstock; stem-pitting of grapefruit; and stem-pitting of sweet orange. The CTV-D strains have eliminated new plantings using sour orange rootstock and is rapidly eliminating groves planted with sour orange rootstock in Florida. Trees on sour orange exhibiting decline are routinely “pushed” or removed from production, causing major economic losses. Rootstock reactions to CTV-D strains presently found in Florida vary from highly susceptible, resulting in tree decline and death (sour orange and *Citrus macrophylla*), to tolerant, wherein no noticeable effect on tree growth is expressed (Carrizo citrange, Cleopatra mandarin, Swingle citrumelo, etc.), or resistant, wherein the virus cannot multiple in the rootstock (Trifoliate orange). The CTV-D strains cause death of the phloem at the bud union. Phloem death produces a girdling effect which may result in overgrowth of the scion at the bud union, paucity of feeder roots, stunting, yellowing of leaves, reduced fruit size, poor growth, dieback, wilting, and death. Declining trees on sour orange rootstock may also exhibit pin holing or honeycombing on the inner face of the bark, or brown discoloration at the bud union.

However, the most virulent and damaging CTV strains are those causing stem-pitting (deep pits in the wood under depressed areas of bark) in the trunk, branches and twigs of scions regardless of rootstock, resulting in trees with low vigor. Twigs on infected trees are brittle and break easily when intentionally bent or blown by the wind under fruit load. Stem-pitting may not be apparent until the bark is peeled from twigs (Figure 3). Rope-like external symptoms can also be cause by stem-pitting strain in trunks of grapefruit and / or sweet orange scions (Figures 4, 5). Stem-pitting of scions due to CTV results in decline but does not usually cause tree death. The economic impact of these strains results from reductions in fruit set, size, and quality (Figure 6). Stem-pitting strains are transmissible by *T. citricida* but not usually by *A. gossypii, A. spiraecola,* or *T. aurantii.* These CTV strains commonly occur in Asia, Australia, Southern Africa, Brazil, Columbia, Venezuela and other areas.

Stem-pitting strains have not yet been identified in Florida, however a severe strain of CTV was detected in dooryard plantings and commercial plantings of Meyer lemon. The strain from Meyer lemon caused moderate to severe stem-pitting on grapefruit in biological assays. Imported citrus material may harbor CTV strains capable of causing stem-pitting, but their presence may not be obvious if the introduced material is tolerant to the virus, such as lemon or one of the mandarins. There is a possibility that stem-pitting strains of CTV may have been unknowingly imported into Florida before the Florida Budwood Registration Program was established in 1952 by the Division of Plant Industry (DPI) of the Florida Department of Agriculture and Consumer Services, or through illegal importation thereafter. Although such strains have not yet been detected, the movement of such "sleeping" severe strains would be greatly accelerated now that *T. citricida* is present.

Diagnostic Tests

Mexican lime has been the standard indicator (bioassay plant) used to confirm the presence of CTV. However, it is not useful for differentiation of seedling yellows, decline on sour orange, or stem-pitting. The enzyme-linked immunosorbent assay (ELISA) is a rapid laboratory test that detects CTV in plant tissue by means of antibodies reactive with the virus. The use of polyclonal antibodies enables detection of all CTV strains. A more selective monoclonal antibody has recently been developed which detects some severe CTV strains such as those causing decline on sour orange rootstock or stem-pitting.

However, there is currently no rapid method to identify and differentiate the strains that cause stem-pitting on grapefruit and sweet orange. Differentiation of CTV strains relies on graft transmission to a battery of bioassay plants including Mexican lime (reactive to most strains), a sweet orange scion grafted onto sour orange (for seedling yellows), Madam Vinous (for stem-pitting on sweet orange), and Duncan grapefruit (for stem-pitting on grapefruit) seedlings. The biological assays for stem-pitting require 10 to 12 months under controlled greenhouse conditions. Development of additional diagnostic tests to differentiate CTV strains on a more timely basis using strain-specific antibodies, nucleic acid probes, and polymerase chain reaction protocols is under way.

Management

Management strategies for CTV are based on the presence and incidence of the virus and its vectors. **Quarantine** against importation of CTV conducted by DPI is important in preventing the introduction of new severe strains, such as those which cause stem-pitting.

The **Florida Budwood Registration Program** maintains and makes available to nurserymen tested virus-free budwood for use in citrus propagation. Only budwood from trees registered as virus-free should be used for propagation. Unfortunately, budwood trees grown in outdoor increase blocks are largely infected already with CTV and some of the registered sites can no longer provide budwood. Trees grown in screenhouses or with other physical barriers that exclude aphids can ensure virus-free budwood. Currently, five commercial nurseries and two agencies (DPI and the Florida Citrus Foundation) produce virus-free budwood in greenhouses in Florida. Their capacity at present enables only limited quantities of budwood.

Removal of declining trees on sour orange and **replacement** with trees budded on a tolerant rootstock is recommended when a tree is no longer economically viable. Replacement trees should be virus-free. Whether to remove and replace the declining trees yearly or to replace an entire block at once is dependent upon each individual situation. The decision must be consistent with the production goals, economic considerations, and management practices of each unique citrus producer.

Suppression and elimination of severe, stem-pitting CTV strains is extremely important to prevent and delay their distribution. Production managers and grove workers should constantly monitor trees for infection by stem-pitting strains. Trees exhibiting symptoms of stem-pitting should be reported to DPI in Gainesville for confirmation and appropriate action.

Mild strain cross protection may be implemented immediately to help prolong the economic life of groves which are exposed to severe strains of CTV. Mild strains of CTV have been selected which reduce the damage by severe CTV strains on sour orange rootstocks. Joint research with international scientists indicated that some of these same mild CTV strains may provide cross protection against stem-pitting strains of the virus. Intentional introduction of these known and well-characterized mild CTV strains into propagative material would, over a period of several years, result in their widespread prevalence. Proliferation of these mild strains would thus lessen the probability that severe CTV strains could be acquired and transmitted by aphid vectors.

Progress is also being made on the introduction of **virus resistance** into desirable citrus varieties. Immunity to CTV present in *Poncirus trifoliata* has been transferred into sweet orange type plants suitable for use as breeding parents by conventional plant breeding techniques. In addition, cells of commercial citrus varieties have been fused with CTV-resistant but non-sexually compatible relatives by somatic hybridization. This process provides an approach to utilize resistance genes not previously available in citrus. It also is now possible to genetically change (transform) citrus through the introduction of foreign genes. Such genes, often from the virus itself, have been shown to induce resistance to the virus in the transgenic plants. While rapid progress is being made in these areas, promising plants must be evaluated for virus resistance and horticultural performance over a number of years before being used on a commercial scale. These approaches will all aid in the future management of CTV.

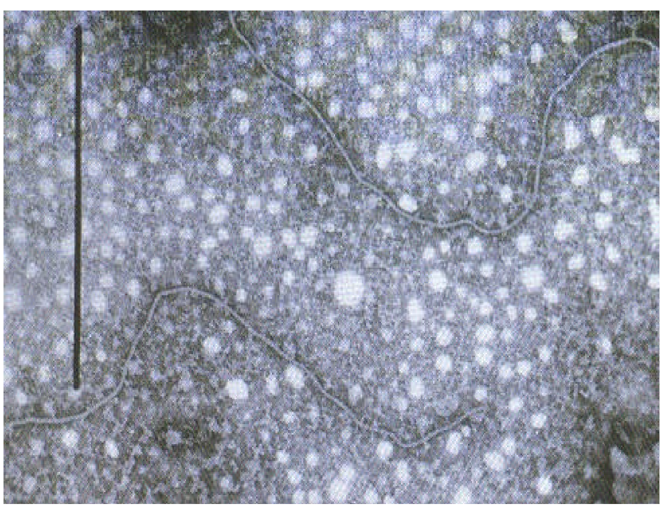

Figure 1. Citrus tristeza virus as seen with a transmission electron microscope after positive staining. The bar indicates 500nm.

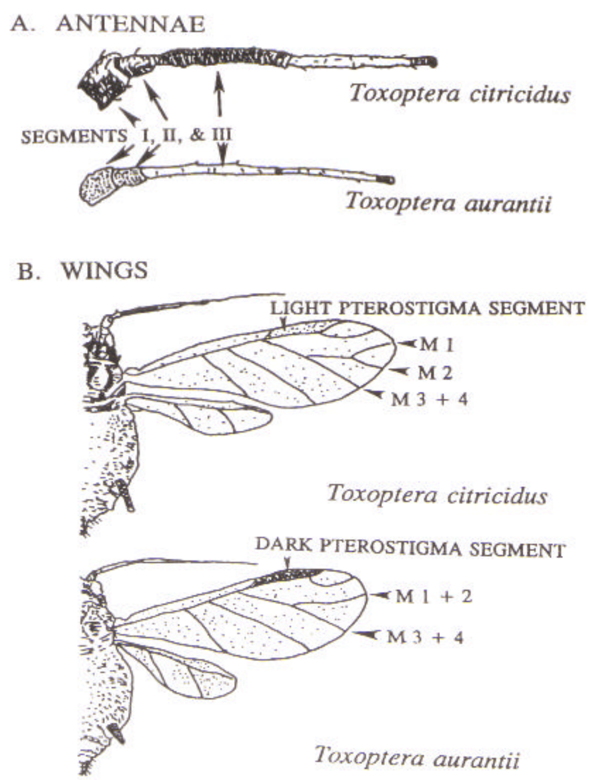

Figure 2. Diagnostic characteristics of *Toxoptera citricida* and *T. aurantii* which permit field identification using a 20x hand lens.

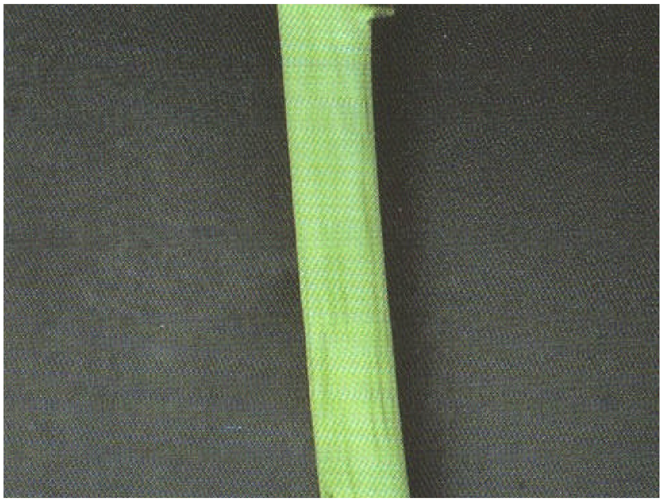

Figure 3. Stem-pitting on small branches of a Pera sweet orange tree in Brazil.

Figure 4. Rope-like external symptoms caused by a stem-pitting CTV strain in the trunk of a grapefruit tree in Australia.

Figure 5. Stem-pitting in a grapefruit tree in South Africa not obvious until a patch of bark was removed.

Figure 6. Small fruit from a grapefruit tree infected with a stem-pitting strain of CTV in Columbia.