Citrus Variegated Chlorosis, A New Severe Disease in Brazil: A Review

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ABSTRACT. Citrus variegated chlorosis was first recogized in the southwestern part of the state of Minas Gerais and in São Paulo state in 1987. It is rapidly spreading and is causing concern to the Brazilian citrus industry. The disease is apparently caused by the xylem-limited bacterium *Xylella fastidiosa*.

A new disease of citrus was observed in Minas Gerais and São Paulo states in Brazil in 1987, and was called citrus variegated chlorosis (CVC) (16, 32). Since then, research by Brazilian workers, with co-operation from researchers in Florida and France has provided some understanding of this new disease, although much still needs to be investigated. The following paper reviews the major findings of this research.

SYMPTOMS

Leaf chlorosis, similar to zinc deficiency or greening disease, begins in the middle and upper part of the tree canopy. Older leaves display conspicuous chlorotic variegation on the upper surface. Analysis showed there to be low levels of both zinc and potassium, even when are readily available (26). The chlorotic spots correspond to small brown blisters on the lower surface. These latter symptoms resemble boron toxicity or molybdenum deficiency (22). The fruit of affected trees are small, somewhat hardened and mature early. Severely affected trees frequently have protruding branches with small leaves and fruit, and a degree of defoliation. Small fruit also occur on branches without foliar symptoms. CVC mainly affects young trees, up to 7-8 yr of age after which they tend to slowly disappear. The symptoms resemble those of "pecosita" in Argentina (6).

Symptoms have been observed on several sweet orange varieties independent of the rootstock used. Pera, Hamlin, Natal and Valencia sweet oranges are the most susceptible. Tangerines and Tahiti lime were symptomless even when growing close to severely affected sweet orange trees.

ECONOMIC LOSSES

Between 1990 and 1992 the effects of CVC on citrus yields were studied in an orchard with intermediate severity (1, 23, 24). Preliminary results show that although the total number of fruit was higher from diseased trees, the mean weight was considerably reduced. Mean loss was estimated to be approximately 20%. Further evaluations are under way.

ETIOLOGICAL STUDIES

Because of the similarity to greening, samples were sent to INRA, Bordeaux, France for examination. No greening BLOs were found, but bacteria were abundant in the xylem of affected trees, while they were absent from healthy samples (31,32,34,35). They were found in leaf midribs, fruit columella, and petioles, and resembled *Xylella fastidiosa* (32). These observations were confirmed both in Brazil (8) and the USA (15). Treatment of infected trees with oxytetracycline resulted in remission of symptoms in one out of ten treated trees after 6 months, which could indicate the involvement of a procaryotic organism (7). Strains of *X. fastidiosa*, which was first formally described in 1987 (38), have been found to be pathogenic in other plants causing serious diseases (11,14). In af-

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fected citrus, the bacterium measures 1 to 3.5 μm by 0.3 to 0.5 μm, and the rippled cell wall composed of three layers, thus resembling the bacteria in other hosts (8).

In 1982, the causal organism was isolated from diseased grapevine (12) and plum (27). In 1991 *X. fastidiosa* was first isolated from CVC-affected tissue in BCYE medium, and these isolates reacted positively in ELISA tests for the bacterium (20,21). Subsequent isolations in PW medium and PW modified with L-histidine (PWH) were made from leaf midribs, petioles and stems of 4-yr-old trees (18). Several isolates (28) of the CVC organism were compared by polyacrylamide gel electrophoresis for total proteins, but no differences were deteted (28). The bacterium has now been isolated on several media, namely modified PW, CS 20, PD 2, CVC 1 and CVC 2 (9,10), and has been isolated from symptomless trees (2). Recently, Koch's postulates were fulfilled both by the Florida and France groups (10,15).

SEROLOGY

The successful isolation of the bacterium made the production of antisera against it a simple matter, and several reports for its detection in citrus tissues by serological methods have appeared (3,4,9,10,13).

Lee *et al.* (17) developed a dot-immunobinding assay (DIBA) using a polyclonal antibody preparation. Serological comparisons were made between the cultured CVC organism and *X. fastidiosa* from other diseased plant species. Strong reactions with antisera to periwinkle wilt and phony peach bacteria were reported (17). In Bordeaux, DSA-ELISA assays using CVC antisera gave high OD readings with several *X. fastidiosa* strains, especially those from grapevine affected with Pierce's disease and almonds with leaf scorch (9).

ELISA assays conducted in Brazil with fresh plant material also gave positive results with both symptomatic and asymptomatic leaf samples from same branch, whereas root tissues of symptomatic trees, leaf tissues of asymptomatic trees in affected orchards and trees in unaffected areas were all negative (13).

TRANSMISSION STUDIES

CVC has spread rapidly through São Paulo, Paraná, Goiás states, possibly by insect vectors. Cicadellids are known vectors for *X. fastidiosa* in other hosts (14). These insects are abundant in citrus orchards on trees, grasses and other native plants (19, 37).

Transmission experiments in the field gave indirect indications of the involvement of vector insects, since CVC symptoms were not found on plants protected against insects in 13-14 months (36) while the unprotected plants with same treatments showed conspicuous symptoms. Graft transmission trials were conducted in the field using blind buds and side-grafts on field trees. Insertion of infected portions of leaf, fruit, seeds and roots into stems was tried in healthy glasshouse plants. In 1898 blind bud transmission using buds with a portion of wood was obtained (31, 33). These results were not repeated in more recent trials with commonly used buds (36). Transmission using pure cultures of the CVC bacterium has also been successful (9, 10, 15).

Small seeds from the small fruits of affected trees give rise to seedlings which contain the CVC organism, but they are symptomless. Seedlings from normal seeds do not contain the bacterium (29).

VARIETAL RESISTANCE

In one experiment buds of 17 varieties, 6 sweet oranges, two each of tangerines, tangerine hybrids, grapefruit and limes, and one lemon were grafted onto the branches of CVC-affected trees. So far after 10 months only the sweet orange shoots are displaying symptoms (36).

In another trial conducted by Pompeu (personal communication), a larger number of varieties are being tested, but no results are yet available.

CONCLUSIONS

CVC is a serious new disease of young citrus trees in Brazil. It is caused by strains of the bacterium, *X. fastidiosa*, and it is spreading rapidly in the state of São Paulo, possibly by cicadellids. Serological tests are now available to test citrus nursery material to prevent the spread of the disease in new trees.

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