A New Type of Decline on Citrus Trees in Brazil

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A NEW TYPE of citrus decline was observed in the Araraquara region of the state of São Paulo in August 1967. It occurs on 3–7-year-old nucellar-clone trees of all the sweet orange varieties cultivated in the area—all on Rangpur lime rootstock, the only rootstock used there. The varieties affected are Baianinha, Hamlin, Westin, Valencia, Natal, and Pera.

On a visit to Tucumán, Argentina, the senior author, in company with Dr. J. L. Foguet, observed identical symptoms on 4–5-year-old trees of sweet orange and tangerine varieties growing on Rangpur lime (2).

The present paper reviews the symptomatology of the new type of decline and reports the results of studies on transmission and chromatography.

Symptomatology

Symptoms are most conspicuous during the dry winter months. At Araraquara the mean winter temperature is 22 °C, with extremes of 3 °C and 24 °C. The winter rainfall is 100 mm.

The major symptoms are briefly: 1. stunting and decline of trees (Fig. 1); 2. leaf vein banding and chlorosis; 3. rotting of roots and rootlets, the rot starting at the root apex but not affecting the trunk; 4. severe stem pitting typical of that of tristeza in branches of all varieties, the branches being easily broken (3); 5. inverse pitting and honeycombing in the trunk and branches of the scion, mostly linearly dispersed (Fig. 2); and 6. discoloration of the wood of the Rangpur lime rootstock (4, 6).

A conspicuous yellowing of the midrib gradually extends to veins and veinlets, including a narrow strip of leaf tissue surrounding the veins, while the remainder of the leaf stays green. Later the leaves become unevenly chlorotic, with rare patches of dark green. Defoliation can seriously damage 4–6-year-old trees. In some cases, symptoms, including defoliation, begin on one side of the tree, but little by little the entire tree is affected. In 7–8-year-old trees, a well-developed branch in the upper part of the tree may show foliar symptoms, which may be the first sign of the disease. The foliar symptoms displayed by diseased trees are typical of those caused by diseases that affect the root system; they are always found in declining trees in winter but may disappear partially or completely at the onset of the warm rainy season. The cycle of severe symptoms in winter followed by recovery in summer may continue for several years until the tree dies.

FIGURE 1. *A 5-year-old tree of a nucellar clone of Baianinha sweet orange showing severe symptoms of decline.*

In some respects, the symptoms of the new type of decline are similar to those of tristeza—the rotting of the roots, the vein banding, the severe pitting, and the easily broken branches. Moreover, Kitajima found a significantly higher number of virus particles thought to be those of tristeza in electron microscope preparations from trees with decline than are usually found in connection with severe strains of tristeza virus. On the contrary, the iodine test with declined trees was negative, indicating no interference with translocation of carbohydrates from scion to stock.

In some respects also, symptoms of decline are similar to those of greening or stubborn. The fact that a vigorous branch may show leaf symptoms before the rest of the tree does, the facts that symptoms are conspicuous during periods of low temperature and disappear when the temperature becomes high, and the difficulty of transmitting the disease by budding are reminiscent of greening as it occurs in Africa and Asia. On the other hand, the rotten roots, inverse pitting, and stem-pitting symptoms associated with decline are not characteristic of greening. Moreover, neither E. W. Kitajima in Campinas nor J. M. Bové in Versailles found mycoplasma by electron microscopy in diseased specimens sent to them by the senior author.

Distribution

The new decline has thus far been found in Brazil only in 11 orchards in Araraquara—the majority of which are located in or near Cabeceira do Boi—and in 2 orchards in Santa Lucia. Of a total of about 145,000 trees in the orchards mentioned above, approximately 700 showed symptoms (4). The soil in the region is variable, but many of the orchards are on poor sandy soil and are not properly fertilized. The disease is most prevalent on trees 3-6 years old but may occasionally be found on trees as old as 12 years. The most severely affected orchard was carefully surveyed, at intervals in 1967. In the first survey, approximately 5 per cent of the trees were found to be affected; in a survey made 6 months later, approximately 9 per cent of the trees had symptoms of decline (6). The spread was slower in other orchards.

FIGURE 2. *The trunk of a Baianinha sweet orange tree on Rangpur lime with bark removed to show a linear disposal of inverse pits extending to the branches.*

Transmission Trials

The symptomatology of the decline and its slow rate of spread suggest that it may be a virus disease transmitted by an insect vector. It has been said that it may be caused by a strain of tristeza virus that attacks trees on Rangpur lime rootstock. A fungus of the genus *Botryodiplodia* has been consistently isolated from the rotten root tissues; other fungi have been isolated less frequently (6). According to a newspaper report, Lordello found the nematode *Pratylenchus brachyurus* (Godfrey, 1929) Goodey, 1951 associated with the disease.

Transmission of decline was attempted by 1. budding or side grafting greenhouse-grown healthy plants with budwood or small branches from severely diseased trees or by a combination of budding and side grafting; 2. topworking branches of healthy trees in the field with budwood from diseased trees; 3. inserting large pieces of bark from affected branches into the trunk and branches of healthy trees in the field; 4. infesting healthy test plants in the greenhouse with *Toxoptera citricidus* (Kirk.) and *Diaphorina* sp. taken after they had fed on diseased trees; 5. inoculating roots of healthy trees in the field and of young plants in the greenhouse with the fungi isolated from rotten roots; and 6. planting young healthy trees in soil taken from around declining trees.

Symptoms of decline did not appear in any of the test plants inoculated in the various ways enumerated above nor were symptoms perpetuated in buds from diseased branches that were allowed to grow out on healthy test plants. Eight of the budded test plants were sent to Dr. J. M. Bové of Versailles to be grown at various temperatures in comparison with young plants infected with the pathogens of greening and stubborn; he reported that no symptoms developed in the budded plants at any temperature to which they were exposed.

Chromatographical Studies

Extracts of the albedo from fruit of affected and nonaffected trees were chromatographed according to the methods of Schwarz (7) in order to determine whether or not a marker substance comparable to that characteristic of greening could be found. Extracts of fruit albedo from both healthy and greening-diseased

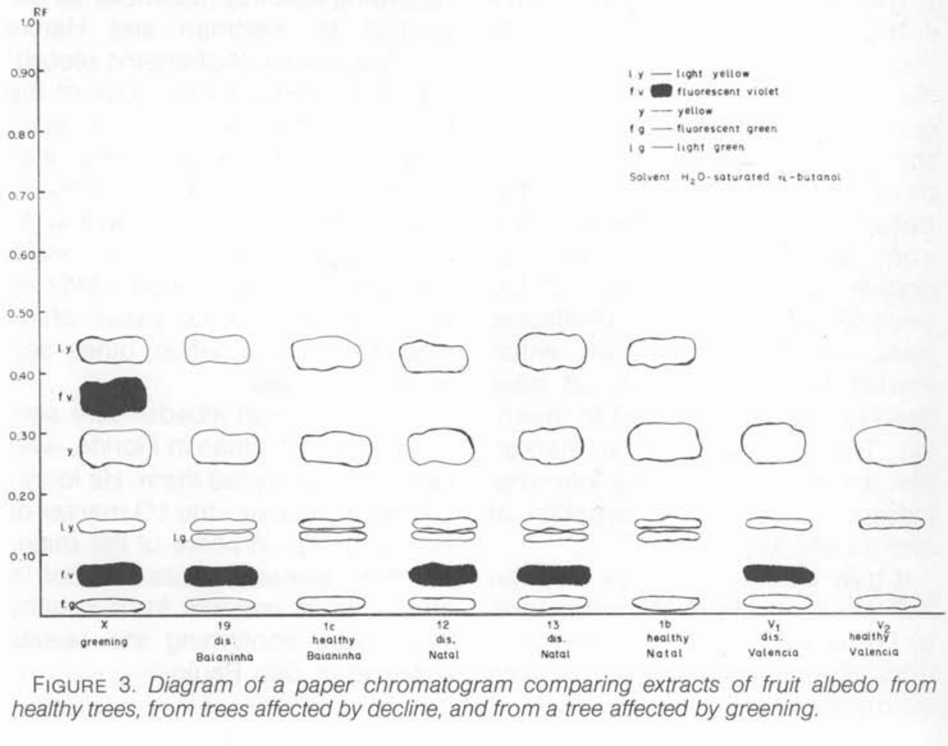

FIGURE 3. *Diagram of a paper chromatogram comparing extracts of fruit albedo from healthy trees, from trees affected by decline, and from a tree affected by greening.*

TABLE 1. OCCURRENCE OF FLUORESCENT SPOTS AT RF 0.08 ON PAPER CHROMATOGRAMS OF EXTRACTS OF ALBEDO AND BARK FROM CITRUS TREES AFFECTED BY DECLINE AND FROM HEALTHY TREES

Variety	Tree status	No. trees	Fruit	Branches
Baianinha	diseased	7	2/4[a]	0/29[a]
	healthy	5	0/2	0/22
Natal	diseased	5	20/22	0/24
	healthy	5	0/22	0/21
Valencia	diseased	3	4/11	
	healthy	1	0/4	
Hamlin	diseased	1	2/4	0/2

a. Number of samples showing fluorescent spots/number of samples examined.

trees from South Africa, kindly supplied by Dr. Schwarz, were used for comparison. Both thin layer plates (Merck) and Whatman No. 1 paper were employed, the chromatograms being sprayed with sodium borate and examined under an ultraviolet lamp radiating mainly at 366 nm.

The data are summarized in Table 1 and Figure 3. A bright blue violet fluorescent spot, corresponding to the greening marker 1G, was frequently found at Rf 0.08 in preparations from the albedo of fruit from diseased trees but not from healthy trees, or in preparations of bark from diseased trees. Chromatographic analysis of extracts of the veins of leaves from decline-affected trees also failed to reveal either marker 1G or 2G as would have been expected in the case of greening. The second greening marker, 2G, did not appear in the chromatographic profiles of extracts of decline-affected trees (5).

It may be worthwhile to mention that recent chromatographic studies by Ferreira de Mello, De Conti, and Rossetti (not yet published) revealed the presence of a fluorescent spot at Rf 0.08 in preparations from trees infected with various strains of tristeza virus, especially with the Capão Bonita and Pera strains.

Some of the fluorescent spots at Rf 0.08 obtained from decline diseased trees were eluted with water and analyzed in a Beckman DK2 recording spectrophotometer as described by Feldman and Hanks (1). The spectrophotometric recordings corresponding to some of the decline-affected trees were coincident with those obtained with material from greening-affected trees, but those corresponding to other decline-affected trees were not. Similar results were obtained with two-dimensional paper chromatograms, and when other solvents were used.

Extracts of fruit albedo were sent to Dr. A. W. Feldman in Florida, who very kindly analyzed them. He found gentisoyl glucose—the 1G marker of greening (1)—in some of the material from diseased trees but not in others or in material from healthy trees, thus confirming the results obtained in São Paulo.

Discussion and Conclusions

The finding of gentisoyl glucose in the fruit albedo of decline-affected trees and the absence of the corresponding fluorescent marker in bark of the same trees is contradictory. It is evident that much more work with the decline should be done before definite conclusions can be drawn. This is particularly true considering the fact that the marker was also found in material from sweet orange trees infected with certain strains of tristeza virus.

In order to prevent the spread of the new type of decline to other areas, the Secretary of Agriculture has established certain rules, including prohibiting the movement of propagative material from areas in which the decline occurs to other areas, eliminating all citrus nurseries in the farms where the disease occurs, the recommended destruction of all diseased trees, and a recommendation to growers to apply efficient treatments for control of possible insect vectors. It is too early to assess the effectiveness of these rules in controlling the spread of the new type of decline.

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