A Tristeza Virus Complex Severe to Sweet Orange Varieties

G. W. MÜLLER, O. RODRIGUEZ, and A. S. COSTA

THE MAJORITY of sweet orange [*Citrus sinensis* (L.) Osb.] varieties grown in Brazil are tolerant to tristeza and will develop satisfactorily when budded on tristeza-tolerant rootstocks. Exceptions to this behavior are the Pera sweet orange and Florida seedling varieties (1, 2, 3) which are rather sensitive to certain severe tristeza virus complexes.

The occurrence of a type of decline shown by several sweet orange varieties budded on tristeza-tolerant rootstocks in Capão Bonito County of São Paulo, Brazil, was brought to the writers' attention late in 1965.* Field observations in Capão Bonito County and greenhouse experiments in Campinas with the material collected in that area indicated that this sweet orange decline is caused by a complex of the tristeza virus different from those present in the important citrus-growing areas of São Paulo. The results obtained to date in the studies on the Capão Bonito sweet orange decline, considered as due to a new tristeza virus complex, are described in this paper.

Geographic Distribution

Surveys in the Capão Bonito area indicated that the sweet orange decline is widespread in the county. The neighboring counties, Angatuba, Guapiara, São Miguel Arcanjo, and Itapetininga, were surveyed to a smaller extent, but the sweet orange decline was not recorded in orchards planted in those areas.

*The authors wish to thank Dr. Milton Alcover, Head, Capão Bonito Agricultural Experiment Station, for calling our attention to the problem and for his cooperation in gathering information on the disease.

The virus complex responsible for the Capão Bonito sweet orange decline might also occur in Eldorado County, where severe stem pitting of Rangpur lime (*C. reticulata* var. *austera* hyb.) rootstocks has been noticed for more than 15 years (Moreira, S., and Costa, A. S., personal communications) and was more recently recorded on the Caipira sweet orange rootstock (4).

A sweet orange decline induced by a virus complex has not been recorded in the important citrus growing areas of Araraquara, Bebedouro, and Limeira, except for the Pera decline due to severe tristeza stem-pitting complexes present in these areas.

Reaction of Citrus Types to the Capão Bonito Virus Decline

Field observations made at the Capão Bonito Agricultural Experiment Station and in other citrus groves in the same county indicated that the Capão Bonito decline was affecting practically all sweet orange varieties planted in the area. Decline symptoms were recorded on Baianinha, Hamlin, Maracanã, Piralima, Sanguinea de Mombuca, Caipira, Seleta, and Barão sweet orange. The Pera sweet orange and Galego lime [*C. aurantifolia* (Christm.) Swing.] plants were also affected, but the symptoms were practically the same as when infected with the common severe tristeza complexes.

When Caipira sweet orange and Rangpur lime were used as rootstocks they generally showed stem pitting if the scion was infected, whereas in other areas of the State of São Paulo this was not generally so.

Tangerines and mandarins (*C. reticulata* Blanco), lemon [*C. limon* (L.) Burm.], and sour orange (*C. aurantium* L.) were not affected.

Symptoms

Sweet orange variety tops affected with the Capão Bonito virus complex show a poor rate of growth, abnormally short axillary sprouts, and twigs that break easily when bent (Fig. 1,A). The leaves are smaller than normal and exhibit deficiency-like symptoms. Severe stem pitting is always present and might look somewhat different from the common tristeza pitting symptoms observed on Pera sweet orange in other areas, the pits being more numerous, shorter, and shallower.

The fruits produced on affected sweet orange trees are smaller in size, dry, and have malformed seeds (Fig. 1,B). The average fruit weight of five sweet orange varieties in the Capão Bonito and Limeira areas was 68.2 g and 138.2 g, respectively.

On Pera sweet orange, Galego lime, and Ruby Red grapefruit (*C.*

paradisi Macf.), the symptoms induced by the Capão Bonito complex are not substantially different from those induced by a very severe tristeza source from other areas.

Materials and Methods

Seedlings of sweet orange varieties† or other citrus types used in the tests were raised in the greenhouse. The seedlings used for the aphid inoculations were generally younger than those used for tissue union inoculation. Also, younger plants were used for leaf piece grafts than for budding.

The Capão Bonito virus sources utilized in the transmission experiments were collected from typically affected sweet orange scions growing in the area that showed decline and severe stem-pitting symptoms. The tristeza severe complexes used for comparison purposes are standard virus sources that have been used in experiments carried out at the Virus Department, Instituto Agronômico, Campinas.

Transmission tests by tissue union were made by inserting 1 or 2 buds in the test plants and by leaf piece grafts, using per plant at least 2 rectangles of leaf, about 1 cm x 0.5 cm, from leaves that had been previously washed with a detergent.

Insect transmission tests were carried out with the tropical citrus aphid *Toxoptera citricida* Kirk. reared on squash (*Cucurbita moschata* L.), then transferred and permitted to breed on the desired virus source. The viruliferous aphids were permitted to feed on the test plants for 48 hours, and were then killed with an insecticide spray. A minimum of 15 aphids per plant was used in each test.

TISSUE UNION TRANSMISSION TESTS.—Seedlings of a number of sweet orange varieties; Rangpur lime; Dancy, Cravo, and Cleopatra tangerines; and Eureka lemon were inoculated with the Capão Bonito virus by leaf piece grafts. Comparable seedlings were inoculated with 3 severe tristeza virus isolates from other areas. Three plants of each type were inoculated with each virus source. The results from the tests, 5 months after inoculation, are shown in Table 1. For the Pera sweet orange seedlings, there was little difference between the symptoms induced by the Capão Bonito virus complex when compared with the tristeza complexes, although symptoms tended to be slightly more severe in the first case. For the other sweet orange varieties, the Capão Bonito virus complex induced

†The authors are indebted to Dr. Carlos Roessing, Head, Limeira Agricultural Experiment Station, who kindly supplied most of the citrus seeds used in the present work.

definitely more stunting, decline, and stem pitting than did the severe tristeza complexes. The Rangpur lime seedlings inoculated with the Capão Bonito complex showed stem pitting, but no decline, whereas the tristeza complexes did not induce that symptom. The inoculated mandarin and tangerines did not develop any symptoms.

APHID TRANSMISSION TESTS.—Twenty-day-old seedlings of several sweet orange varieties, Galego lime, Ruby Red grapefruit, and Cravo

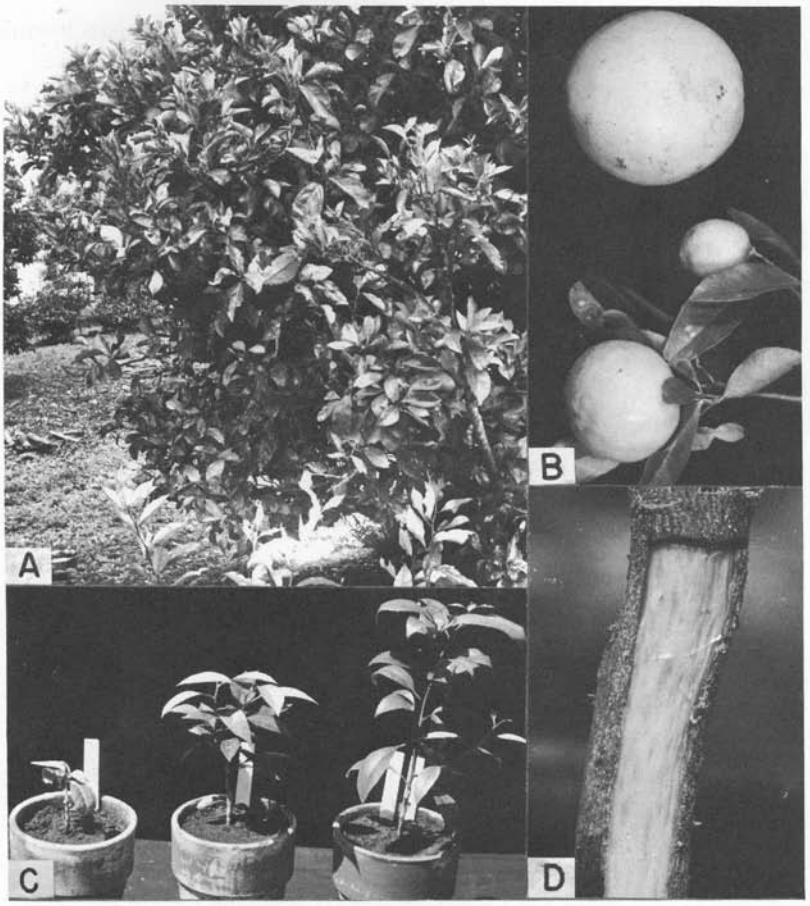

FIGURE 1. *Symptoms of the Capão Bonito decline. A. Seleta sweet orange showing abnormal axillary twigs and yellowing. B. Normal sweet orange (above) and small fruits produced by infected tree. C. Symptoms induced by the Capão Bonito complex (left), tristeza stem-pitting isolate (middle), and check (right). D. stem pitting on Rangpur lime induced by the Capão Bonito complex.*

and Cleopatra tangerines were inoculated by means of the tropical citrus aphid with the Capão Bonito sweet orange decline. Comparable seedlings were inoculated with a severe tristeza complex from a Barão sweet orange source. Five seedlings of each type were inoculated, and the results obtained 5 months after inoculation are shown in Table 2. The Capão Bonito virus complex induced considerably more severe symptoms on the sweet orange varieties than on the Barão complex (Fig. 1,C).

TABLE 1. RESULTS FROM COMPARATIVE INOCULATIONS WITH THE CAPÃO BONITO VIRUS COMPLEX AND 3 TRISTEZA COMPLEXES BY MEANS OF LEAF PIECE GRAFTS[a]

Citrus type tested	Growth (g), decline (s), and stem-pitting (p) scoring for seedlings inoculated with the indicated virus source														
	Capão Bonito			12			120			Barão			Check		
	g	s	p	g	s	p	g	s	p	g	s	p	g	s	p
Sweet oranges															
Pera	2.0	4.0	5.0				2.0	3.0	3.0	2.0	3.0	4.0	5.0	0	0
Caipira	3.5	3.0	3.0	5.0	0	0	3.5	0	0	3.5	0	0	5.0	0	0
Baia	3.0	4.0	5.0	4.5	0	0	4.0	2.0	2.0	4.0	0	0	5.0	0	0
Natal	5.0	0	2.0	4.5	0	0	4.5	0	0	4.5	0	0	5.0	0	0
Seleta	3.0	2.0	3.0	3.0	0	0	4.0	0	0				5.0	0	0
Westin	4.5	3.0	4.0	4.5	0	2.0	4.0	0	1.0	4.0	0	0	5.0	0	0
Valencia	5.0	0	1.0	4.5	0	1.0	5.0	0	0	4.0	0	0	5.0	0	0
Piralima	3.0	3.0	3.0	4.0	0	0	3.0	0	0	3.0	0	0	3.0	0	0
Tangerines															
Dancy	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0
Cravo	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0
Cleopatra	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0
Others															
Rangpur lime	5.0	0	3.0	5.0	0	0	5.0	0	0	5.0	0	0	5.0	0	0

(g) Growth rating, 1 to 5; 1 = poorest growth; 5 = best.

(s) and (p) Tristeza and stem-pitting symptoms rating, 0 to 5. 0 = no symptoms; 5 = most severe symptoms. Intermediate values for intermediate types of symptoms.

a. Figures in the table represent the average of 3 plants.

For Galego lime seedlings there was no difference between the two complexes, but for the grapefruit seedlings the Capão Bonito complex was slightly more severe. The tangerines were not affected.

TESTING FOR THE CAPÃO BONITO COMPLEX.—One of the characteristics that distinguishes the Capão Bonito virus complex from the severe tristeza virus sources is the strong decline of sweet orange varieties induced by the former. Another distinguishing symptom of the Capão Bonito virus complex is its ability to induce strong stem pitting on Rangpur lime rootstocks or on inoculated seedlings of this species. To estab-

lish this point on a wider basis, an attempt was made to compare the reaction induced on Rangpur lime by the Capão Bonito virus complex with that resulting from inoculations with severe tristeza complexes.

Fifty uniform Rangpur lime seedlings were separated in 5 groups of 10 plants each. These were then treated as follows: 1. budded with 2 buds infected with the Capão Bonito complex; 2., 3., and 4. inoculated in the same manner with severe tristeza isolates 12, 120, and 138, respectively; and 5. left untreated, as controls. After the inserted buds had taken, each group was subdivided into 2 subgroups of 5 plants each.

TABLE 2. RESULTS FROM COMPARATIVE APHID TRANSMISSION TESTS WITH THE CAPÃO BONITO VIRUS COMPLEX AND A SEVERE TRISTEZA SOURCE FROM THE BARÃO SWEET ORANGE[a]

Citrus type tested	Growth (g), decline (s), and stem-pitting (p) scoring for seedlings inoculated with the indicated virus source and for the checks								
	Capão Bonito			Barão source			Checks		
	g	s	p	g	s	p	g	s	p
Sweet oranges									
Piralima	1.2	5.0		3.2	1.0	0	4.0	0	0
Barão	2.0	5.0	3.0	4.5	1.2	0	5.0	0	0
Valencia	2.0	4.0	3.7	3.7	1.0	0	4.0	0	0
Caipira	2.0	2.5	2.5	3.6	1.0	0	4.7	0	0
Tangerines									
Cleopatra	4.0	0	0	4.5	0	0	5.0	0	0
Cravo	4.2	0	0	4.0	0	0	5.0	0	0
Others									
Galego lime	1.0	5.0	5.0	1.0	5.0	5.0	5.0	0	0
Ruby Red grapefruit	3.0	3.5	5.0	2.0	2.0	4.0	5.0	0	0

a. Figures in table represent the average of 5 plants.

Five plants in one of the groups were cut back, leaving a stem about 8 in. high with the 2 inserted buds. Four months after inoculation only the Capão Bonito virus complex had induced stem-pitting symptoms on the Rangpur lime seedlings (Fig. 1,D). Also, the plants that had been cut back showed more severe pitting than those left undisturbed. The Rangpur lime seedlings that were not cut back exhibited more noticeable pitting at the base of the stem or in the roots, whereas in the cut-back plants the symptoms could also be seen near the decapitated end of the stem.

INOCULATION OF RUTACEAE, IMMUNE FROM TRISTEZA, WITH THE CAPÃO BONITO VIRUS COMPLEX.—Seedlings of *Poncirus trifoliata* (L.) Raf. and *Severinia buxifolia* (Poir) Ten. were inoculated by budding

with the virus complex responsible for the sweet orange decline in the Capão Bonito area. None of the inoculated plants of these species showed symptoms. Results from the attempts made to recover the virus from these inoculated species back to sweet orange and Galego lime are not yet available.

Discussion and Conclusions

Environmental conditions in the Capão Bonito area are not different enough from other citrus areas of the State of São Paulo to explain the noted differences in behavior of sweet orange varieties there and in other citrus-growing areas, if they were infected with the same tristeza virus complex. Moreover, the transmission tests carried out in the greenhouse at Campinas showed that the Capão Bonito virus source behaved differently, when compared with known severe tristeza complexes as to their effect on seedlings of several sweet orange varieties. These greenhouse experiments indicated that the Capão Bonito virus source contains a component or components not present or associated with the tristeza virus complexes from other areas.

The symptoms associated with the decline disease of the sweet orange varieties in the Capão Bonito area are very similar to those induced on the Pera sweet orange and Florida sweet seedling by severe tristeza virus isolates from other areas of São Paulo. This resemblance of symptoms and the fact that sweet orange decline of Capão Bonito is transmitted by the same vector as tristeza suggest that the component or components responsible for the sweet orange decline in the Capão Bonito area belong to the tristeza complex and are more injurious to sweet orange tissues. The possibility that the Capão Bonito decline could be caused by a mixture of the tristeza virus and a different virus transmitted by the same vector is not entirely eliminated.

Sweet orange decline seems to be at present restricted to certain regions of the State of São Paulo. It is a limiting factor for the sweet orange production in those areas and a threat to the citrus industry in other parts of the country. Quarantine measures might delay or prevent its distribution to other areas, but its potential importance justifies an intensive program to develop control measures for the disease.

Eradication of sweet orange decline from the Capão Bonito area is, practically speaking, an impossibility. As for the other types of tristeza, control measures have to be developed that will be operative in the presence of the virus. On this basis, the following approaches seem more promising: 1. pre-immunization of virus-free new clones of the sweet

orange varieties with tristeza mild isolates or complexes that will protect them against the invasion by the Capão Bonito component or complex; 2. obtaining mutants from sweet orange varieties that have tissues tolerant to the Capão Bonito disease; 3. incorporation of high tolerance or immunity to sweet orange varieties by breeding.

This paper results from investigations supported in part by P. L. 480 funds (project S. 3-CR-2).

Literature Cited

1. GIACOMETTI, D. C. 1961. Stem-pitting threat to Brazil citrus. Calif. Citrograph 46(7): 243-244.
2. GRANT, T. J., COSTA, A. S., and MOREIRA, S. 1951. Variations in stem-pitting on tristeza-inoculated plants of different citrus groups. Proc. Florida State Hort. Soc. 64: 42-47.
3. MOREIRA, S. 1960. Um novo problema para nossa citricultura. Rev. Agr. (Piracicaba) 35: 77-82.
4. ROSSETTI, V. 1965. "Tristeza" dos Citros. O Biológico 31: 224.