Further Evidence on Protective Interference in Citrus Tristeza

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THE PROBLEM of reducing losses induced by tristeza disease was solved for most commercial citrus types by the use of tolerant rootstocks. This, however, did not prevent the damage that resulted from the disease in cases of certain scions that possess somewhat intolerant tissues, such as the Pera sweet orange [*Citrus sinensis* (L.) Osb.], West Indian lime types [*C. aurantifolia* (Christm.) Swing.], and grapefruit varieties (*C. paradisi* Macf.). For these citrus types, yield losses may result from infection with certain stem-pitting strains or strain complexes of the tristeza virus.

One possible control method for the losses induced by tristeza on the above-mentioned citrus types is the pre-immunization of virus-free clones with mild strains or complexes that would protect the plants against fur-

ther infection with the severe stem-pitting complexes. Protection interference between components of the tristeza virus complex has been reported from several sources (1, 2, 3, 4, 5, 6).

The investigations reported here were undertaken with the aim of finding adequate mild strains or complexes that would not injure the citrus types more susceptible to stem pitting and at the same time would protect them against the field stem-pitting virus complexes.

Material and Methods

SOURCES OF VIRUS ISOLATES.—Mild virus isolates were collected from various citrus-growing areas of São Paulo and especially from orchards where injury due to the severe stem-pitting complexes was more noticeable. Outstanding trees in orchards of Pera sweet orange, Galego lime, and grapefruit varieties that showed mild or less noticeable symptoms were selected as mild virus sources.* In some instances, paired collections were made from the same orchards, one collection from trees with mild or no symptoms and the other from severely affected plants.

Over 70 mild isolates were collected and from these 45 were included in field exposure tests. From 50 mild isolates tested in the greenhouse, only 13 (10 from Galego lime and 3 from Pera sweet orange) induced symptoms that were mild enough to warrant their use in protective interference trials. These were further tested by super-inoculation with severe stem-pitting isolates.

All virus source plants were established by budding in nursery rows at the Main Agricultural Experiment Station of the Instituto Agronômico, Campinas, Brazil.

FIELD EXPOSURE TESTS.—Three nursery plots—Caipira sweet orange, Cleopatra mandarin (*C. reticulata* Blanco), and Rangpur lime (*C. reticulata* var. *austera* hyb.)—were prepared as rootstocks for the field exposure tests. A total of 45 mild and 5 stem-pitting isolates were inoculated on the rootstocks, each isolate on 5 plants of each rootstock. One month after the inoculation, 5 new virus-free clonal lines each of Pera sweet orange, Galego lime, and Ruby Red grapefruit were budded on the inoculated rootstocks so that each isolate was on each of the 5 clones of the 3 citrus types. Growth of the virus-carrying buds was permitted to reach about 10 in. before being trimmed. The scion growth of the new clones was topped and permitted to develop 3 or 4 branches. When the

*The possibility that some of these outstanding plants could represent mutants with tissues of high tolerance to the disease was recognized and investigated.

nursery plants attained the desirable size, they were transplanted to the field and set 13 feet apart in the row with 17 feet between the rows.

In addition to being exposed to field infection, each stock-scion-isolate combination in the orchard was handled as follows: (a) the first 3 plants in the row were kept without further treatment, exposed only to natural infection; (b) the fourth plant of each combination was super-inoculated with 3 buds carrying a challenging stem-pitting isolate (challenger No. 1); and (c) the fifth plant in the row was super-inoculated with the challenging stem-pitting isolate by means of a minimum of 50 aphids (*Toxoptera citricida* Kirk.) bred on the infected virus source (challenger No. 2).

GREENHOUSE TESTS.—The protective effect of mild isolates was evaluated in greenhouse tests by inoculation of small seedlings with the first virus, followed by super-inoculations with the challenging isolate 2 months later. The protective inoculation was made with non-viruliferous aphids from squash that were transferred onto caged young shoots of the virus source plants and permitted to breed. They were then used for inoculation of the very young seedlings. The challenge inoculation was carried out likewise. Healthy controls, as well as healthy controls that were inoculated with the challenging virus only, were prepared.

In preparing blends of mild strains, the components of each blend were inoculated simultaneously by means of 2 leaf pieces each on seedlings of all citrus types to be tested. Six months after the simultaneous inoculation, leaf pieces from the plants that had received the 3 isolates and probably had them in a stable blend were used to pre-immunize seedlings of Pera sweet orange, Galego lime, and Ruby Red grapefruit that were subsequently challenge-inoculated with the stem-pitting isolates.

Experimental Results

FIELD EXPOSURE TESTS.—Three years after the protective inoculation and one and one-half years after planting in the orchard, the effect of 45 mild isolates on the Pera sweet orange and Ruby Red grapefruit scions was practically indistinguishable from the non-inoculated controls. Trees of Pera sweet orange and Ruby Red grapefruit that had been inoculated with only the stem-pitting isolates at the same time when the others were pre-immunized showed only a slightly slower growth and some stem-pitting symptoms. The differences are, however, too small to be reported at present. On the other hand, the reaction of the different isolates on the Galego lime scions was very clear-cut. Out of 45 mild isolates that were used for the protective inoculations, 37 induced me-

dium to severe tristeza symptoms and could not be considered as adequate for protection of this citrus type. Eight of the mild isolates permitted the Galego scions to develop satisfactorily, their type of growth being almost equivalent to that of non-infected control plants. The scoring for growth, tristeza symptoms, and stem pitting of the Galego lime plants pre-immunized with the 8 isolates and further inoculated with the challenging isolates, as well as the respective controls, is shown in Table 1.

GREENHOUSE PROTECTION TESTS OF SINGLE MILD ISOLATES.—A number of mild isolates collected from Pera sweet orange and Galego lime

TABLE 1. FIELD REACTION OF PRE-IMMUNIZED GALEGO LIME SCIONS AFTER SUPER-INOCULATION WITH SEVERE STEM-PITTING ISOLATES

Isolate number	Decline symptoms (s), stem-pitting (p), and growth (g) scoring of Galego lime scions treated as indicated								
	Pre-immunized[c]			Pre-immunized plus challenger No. 1[d]			Pre-immunized plus challenger No. 2[d]		
	s	p	g	s	p	g	s	p	g
Mild 40[a]	1.0	1.0	4.8	1.0	0.6	4.5	1.0	0	4.7
42	1.0	0.7	4.7	1.0	0.3	4.9	1.0	0	4.2
50	1.0	0.7	4.7	1.0	0	4.4	1.0	0.3	4.7
72	1.0	1.0	4.1	1.0	0.5	4.0	1.0	0.5	4.2
128	1.2	1.3	4.3	1.0	1.3	4.4	1.0	1.0	1.0
130	1.0	1.1	3.9	1.0	1.0	4.2	1.0	1.0	4.5
141	1.0	1.0	4.1	1.0	1.0	4.0	1.0	1.0	4.0
142	1.0	1.0	4.2	1.0	1.0	4.7	1.0	1.0	4.2
AVERAGE	1.0	1.0	4.3	1.0	0.7	4.4	1.0	0.6	4.3
Stem pitting 33[b]	4.0	4.4	2.0						
83	3.7	4.8	2.2						
94	4.0	3.2	1.9						
135	4.3	4.8	1.7						
AVERAGE	4.0	4.3	1.9						
Checks, natural infection	2.9	3.5	3.2						
Checks, inoc. challenger No. 1				2.5	2.5	2.6			
Checks, inoc. challenger No. 2							2.5	2.7	3.2
Checks, not infected	0	0	5						

a. Mild isolates used for pre-immunization. Isolate numbers in accession book in the Virus Department.

b. Stem-pitting isolates inoculated on controls at the time other plants were pre-immunized.

c. and d. Figures represent averages based on 9 and 3 plants, respectively.

(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.

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

orchards tested in the field were also compared as to their protective value in greenhouse tests. Thirteen of the best mild isolates have been tested so far on Galego lime, Pera sweet orange, and Ruby Red grapefruit seedlings. The results obtained are summarized in Tables 2, 3, and

TABLE 2. GREENHOUSE COMPARISON OF THE PROTECTIVE EFFECT OF SELECTED MILD TRISTEZA VIRUS INOCULATION ON GALEGO LIME SEEDLINGS SUBSEQUENTLY SUPER-INOCULATED WITH SEVERE STEM-PITTING SOURCES

Isolate number and origin		Tristeza symptoms (s), stem pitting (p), and growth (g) scoring for Galego lime seedlings inoculated in the indicated manner								
		Pre-immunized[a]			Pre-immunized and challenger[b]			Challenger only[b]		
		s	p	g	s	p	g	s	p	g
Mild Pera sweet orange	19	5.0	5.0	1.0						
	28	5.0	5.0	1.0						
AVERAGE		5.0	5.0	1.0				5.0[c]	5.0[d]	1.0[e]
Mild Galego lime	34	2.0	3.0	2.5	2.0	5.0	2.5			
	36	1.0	1.0	4.5	1.0	1.0	4.0			
	38	1.0	1.0	4.7	1.6	2.0	3.3			
	40	1.0	1.0	4.5	2.0	1.0	3.7			
	42	1.0	1.0	4.2	1.0	1.0	3.7			
	50	2.3		2.3	3.5		2.0			
	100	1.5	1.0	3.0	3.5		2.0			
	128	2.0	1.0	2.7	4.0	3.0	1.4			
	141	1.2	1.0	3.5	2.0	3.0	3.0			
	142	1.0	1.0	4.5	1.0	3.0	3.7			
AVERAGE		1.4	1.2	3.6	2.2	2.3	2.9	5.0[c]	5.0[d]	1.0[e]
Stem pitting	83	5.0	5.0	1.0						
	94	5.0	5.0	1.0						
AVERAGE		5.0	5.0	1.0						
Controls (not inoculated)		0	0	5.0						

a. and b. Figures represent averages based on 3 and 5 plants, respectively.
c., d., and e. Scoring for control plants inoculated with the challenger at the same time as the pre-immunized plants.

4. They show that the pre-immunization of Galego lime seedlings with the mild isolates from Pera sweet orange induced symptoms already severe, indicating that they were not adequate to pre-immunize this species. Mild isolates from Galego lime plantings gave somewhat contradictory results, especially when the data from the greenhouse tests were compared with those obtained from the field tests. Although the pre-immunized Galego seedlings, subsequently challenge-inoculated, showed

weaker symptoms than those that received only the challenger, there was an increase in symptoms following the super-inoculation that could be assessed by comparison with those seedlings that were only pre-immunized. Satisfactory protection was noted only in the case of isolates 36, 40, and 42 (Fig. 1,F).

TABLE 3. GREENHOUSE COMPARISON OF THE PROTECTIVE EFFECT OF SELECTED MILD TRISTEZA VIRUS INOCULATION ON PERA SWEET ORANGE SEEDLINGS SUBSEQUENTLY SUPER-INOCULATED WITH SEVERE STEM-PITTING SOURCES

Isolate number and origin		Tristeza symptoms (s), stem-pitting (p), and growth (g) scoring for Pera sweet orange seedlings inoculated in the indicated manner								
		Pre-immunized[a]			Pre-immunized and challenger[b]			Challenger only[b]		
		s	p	g	s	p	g	s	p	g
Mild Pera sweet orange	19	1.5	1.0	3.5	2.0	1.0	3.0			
	28	1.0	1.0	4.3	1.0	1.0	4.0			
	66	1.0	2.0	3.5	1.5	5.0	3.0			
AVERAGE		1.2	1.3	3.7	1.5	2.3	3.3	4.0[c]	5.0[d]	2.0[e]
Mild Galego lime	34	1.1	0	3.5	1.5	0	3.0			
	36	2.0	0	3.0	2.0	2.0	3.0			
	38	1.5	0	3.0	1.5	1.0	3.0			
	40	1.2	0	3.8	1.5	0	3.2			
	42	1.2	0	4.0	1.5	1.0	4.0			
	50	1.0	0	4.3	1.0	0	3.7			
	100	1.7	0	2.1	1.7	0	1.7			
	128	1.0	0	4.0	1.0	0	4.0			
	141	1.0	0	4.0	1.5	5.0	3.0			
	142	1.0	0	4.5	1.0	1.0	4.0			
AVERAGE		1.3	0	3.6	1.4	1.0	3.3	4.0[c]	5.0[d]	2.0[e]
Stem pitting	83	3.0	4.0	2.0						
	94	5.0	5.0	1.0						
AVERAGE		4.0	4.5	1.5						
Controls (not inoculated)		0	0	5.0						

a. and b. Figures represent averages based on 3 and 5 plants, respectively.
c., d., and e. Scoring for control plants inoculated with the challenger at the same time as the pre-immunized plants.

Pera sweet orange seedlings protected with mild isolates from Pera and later super-inoculated behaved identically to those that had been protected with mild isolates collected from Galego lime orchards. When compared with the plants that received only the challenger, there was a good indication of protection (Fig. 1,E).

Ruby Red grapefruit seedlings protected with mild isolates from Pera sweet orange showed stronger symptoms than those that received mild

isolates from Galego lime. Both groups of isolates, however, gave definite protection against super-inoculation with the severe stem-pitting isolates (Fig. 1,G).

MILD ISOLATE BLENDS VERSUS INDIVIDUAL COMPONENTS.—What have been called mild tristeza isolates in the present paper represent individual field strains or strain complexes of the virus collected from outstand-

TABLE 4. GREENHOUSE COMPARISON OF THE PROTECTIVE EFFECT OF SELECTED MILD TRISTEZA VIRUS INOCULATION ON RUBY RED GRAPEFRUIT SEEDLINGS SUBSEQUENTLY SUPER-INOCULATED WITH SEVERE STEM-PITTING SOURCES

Isolate number and origin		Tristeza symptoms (s), stem-pitting (p), and growth (g) scoring for Ruby Red grapefruit seedlings inoculated in the indicated manner								
		Pre-immunized[a]			Pre-immunized and challenger[b]			Challenger only[b]		
		s	p	g	s	p	g	s	p	g
Mild Pera sweet orange	19	2.5	3.6	2.5	2.5	4.0	2.5			
	28	2.0	3.0	2.9	2.0	3.0	2.2			
AVERAGE		2.2	3.3	2.7	2.2	3.5	2.3	5.0[c]	5.0[d]	1.0[e]
Mild Galego lime	34	1.0	0	3.5	1.0	0	3.0			
	36	1.0	0	4.0	1.5	0	3.5			
	38	1.0	0	4.5	1.9	0	3.5			
	40	1.0	0	4.0	1.0	0	4.0			
	42	1.0	0	4.2	1.0	0	3.8			
	50	1.0	0	3.5	1.0	0	3.5			
	100	1.6	0	3.5	1.3	0	3.6			
	128	1.8	0	2.6	3.4	0	1.5			
	141	1.0	0	4.3	1.0	4.0	4.0			
	142	1.5	0	3.0	2.0	3.0	3.0			
AVERAGE		1.2	0	3.7	1.5	0.1	3.3	5.0[c]	5.0[d]	1.0[e]
Stem pitting	83	5.0	5.0	1.0						
	94	5.0	5.0	1.0						
AVERAGE		5.0	5.0	1.0						
Controls (not inoculated)		0	0	5.0						

a. and b. Figures represent averages based on 3 and 5 plants, respectively.

c., d., and e. Scoring for control plants inoculated with the challenger at the same time as the pre-immunized plants.

ing trees in orchards that were severely affected by stem pitting when the collection was made.

In the course of the present work the possibility was examined that the protective effect of mild tristeza virus isolates could be enhanced by adequately blending them. It was also expected that by mixing mild isolates from different citrus areas, a combination could be obtained that would have a wider protection range. The use of blends for protection

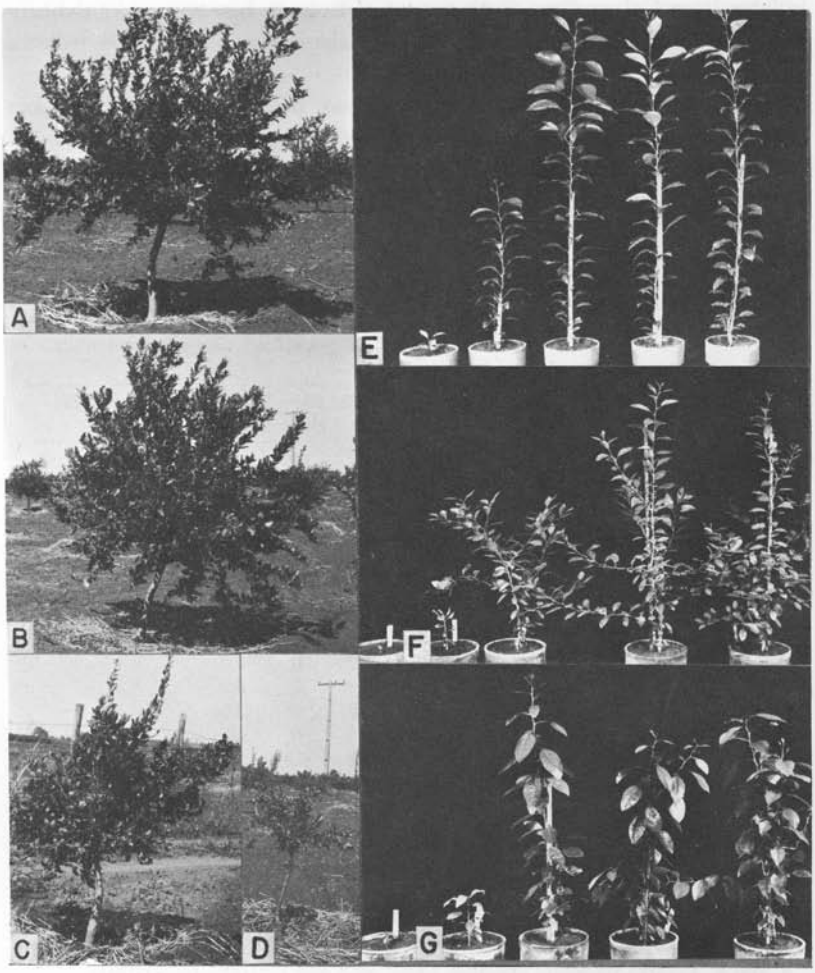

FIGURE 1. *Field interference test. A. Pre-immunized Galego lime plant. B. Pre-immunized and super-inoculated with stem-pitting isolate. C. Non-pre-immunized and naturally infected. D. Control plant inoculated with stem-pitting isolate at the time of pre-immunization. Greenhouse interference test. E., F., and G. Five seedlings each of Pera sweet orange, Galego lime, and Ruby Red grapefruit inoculated (left to right), as follows: (1) with stem-pitting isolates at pre-immunization; (2) with stem-pitting isolates at super-inoculation; (3) pre-immunized with mild isolate and super-inoculated with stem-pitting isolate; (4) with pre-immunizing isolate only; and (5) virus-free controls.*