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# Interaction Studies Between a Mild Strain of Tristeza on Acid Lime and Other Virus or Viruslike Diseases of Citrus

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Mild-strain protection to overcome the effect of severe strains of tristeza virus on acid lime is recommended under normal orchard conditions (Balaraman and Ramakrishnan, 1977; 1978). The possible protection of preinoculated acid lime seedlings when planted in existing orchards where severe tristeza strains and their aphid vector, *Toxoptera citricida* (Kirk.), are present has been demonstrated. However, information on the effect of other viruses or viruslike diseases on mild-strain-inoculated plants is not available. Hence, an experiment was carried out to study the interaction of greening, infectious variegation, citrus mosaic, and woody gall-vein enation with a severe strain of tristeza on preinoculated acid lime seedlings; the results are presented here.

## MATERIALS AND METHODS

Acid lime seedlings raised under greenhouse conditions were used. Pure cultures of pathogens were maintained on their respective indicator plants, viz., greening on sweet orange, infectious variegation on Kinnow mandarin, woody gall-vein enation on rough lemon, mosaic on sweet orange, and tristeza strains on acid lime seedlings. Pathogens were transmitted by a bark-patch method. Treatments were: (i) uninoculated control; (ii) seedlings inoculated with a mild strain of tristeza; (iii) seedlings inoculated simultaneously with a mild strain of tristeza and one of the other pathogens; (iv) seedlings previously inoculated with a mild strain of tristeza and, after symptom development (usually 8 weeks later), challenge-inoculated with different pathogens separately; and (v) seedlings inoculated singly with the respective pathogens. Treatments were replicated three times.

Seedling height, stem diameter, and visual symptoms were recorded regularly for 12 months and data analyzed statistically.

## EXPERIMENTAL RESULTS

The seedlings inoculated with different pathogens did not show any significant difference in their growth rate for up to 3 months (table 1). However, significant differences were noted from the 6th to 12th month. The growth rates of healthy and mild-strain-inoculated plants were similar and comparable with those plants inoculated with greening, infectious variegation, mosaic, and woody gall-vein enation either alone or in combination with the mild strain. No significant differences were noted between the different treatments and all were comparable with the healthy plants (fig. 1). However, the plants inoculated with the severe strain of tristeza alone and simultaneously inoculated with the mild and severe strains ceased growth. The plants were very stunted and showed no significant increase in growth rate after 6 months, whereas the plants in all other treatments grew significantly.

Similarly, there was a steady and uniform increase in stem diameter on all plants, except those inoculated with tristeza alone or inoculated simultaneously with mild and severe strains of tristeza.

Acid lime seedlings inoculated with greening alone showed slight leaf-mottling symptoms but no stunting. Likewise, plants inoculated with the mild strain of tristeza and greening simultaneously, and 8 weeks later, showed no synergistic effect, but only mild vein flecking, slight interveinal chlorosis, and leaf mottling. Apparently, the greening pathogen had no ill effect

on acid lime, either alone or in combination with the mild strain of tristeza.

Similarly, mosaic and infectious variegation showed no external symptoms on acid lime. Acid lime seems to be resistant to, or tolerant of, these pathogens. Concurrent inoculation of the mild strain of tristeza and mosaic and infectious variegation either simultaneously or 8 weeks later produced only mild vein flecking.

Twelve weeks after inoculation, plants inoculated with the woody gall-vein enation virus, either alone or in combination with the mild strain of tristeza, developed enations on the veins on the undersurface of leaves and gall-like structures near the thorns. The presence of the mild strain of tristeza neither prevented nor aggravated the symptoms of the other pathogen. Plants inoculated with the severe and with the severe plus mild strains simultaneously showed severe vein flecking and deep stem pits.

Root and shoot development (table 2) was least in plants inoculated with the severe strain and the severe plus mild strains simultaneously. However, all the other treatments increased significantly in root and shoot weight, and were comparable with healthy and mild-strain-inoculated plants. Girth measurements showed a similar pattern, and no significant differences were noted in these treatment combinations when compared to healthy and mild-strain-inoculated seedlings.

## DISCUSSION AND CONCLUSION

Generally, unrelated viruses capable of infecting and multiplying independently interact when together in the same plant. Infection by one virus may increase or decrease the intensity of symptoms in the presence of a second virus irrespective of sequence of inoculation. Bawden and Kassanis (1945) showed that multiplication of potato virus Y or *Hyocymus* virus 3 was prevented in tobacco plants infected with severe etch virus. McWhorter and Price (1949) reported that two unrelated viruses, tobacco mosaic virus and

tobacco etch virus, could co-exist in the same cell without synergistic or antagonistic action.

In the present study, combined inoculation of acid lime seedlings with a mild strain of tristeza and other pathogens; viz., greening, infectious variegation, mosaic, woody gall-vein enation and a severe strain of tristeza, either simultaneously or 8 weeks later, showed that greening and woody gall-vein enation expressed their respective symptoms along with a mild reaction to tristeza, so apparently either greening or woody gall-vein enation caused interference. These pathogens might co-exist with tristeza virus without any synergistic action. Mosaic and infectious variegation, either alone or in combination with a mild tristeza strain, on acid lime did not express external symptoms.

The mild strain of tristeza virus completely protected against the severe strain when challenge inoculation was done 8 weeks after the protecting inoculation, confirming an earlier report (Balaraman and Ramakrishnan, 1977). Simultaneous inoculation of mild and severe strains caused a severe reaction, indicating the dominant nature of the severe strain. Weathers (1960) reported that inoculation of West Indian lime with citrus psorosis when yellow vein virus was already present did not affect yellow vein symptoms, but the reverse resulted in symptoms similar to those of simultaneous inoculation. Further, dual infections with yellow vein and vein enation viruses increased the severity of symptoms in lime, lemon, and sweet orange seedlings. Synergistic reactions were reported between mosaic and yellow corky vein (Murti and Reddy, 1976), yellow vein and wood gall (Weathers and Greer, 1967), psorosis and exocortis (Weathers *et al.*, 1969), and greening and seedling yellows (Martinez, 1972a, b). Grasso (1973), however found that simultaneous infection by ringspot, infectious variegation, and psorosis A viruses on lemon plants apparently did not interfere with symptom expression of any individual virus.

The present study indicated that acid lime is tolerant of mosaic, infectious variegation, greening, and woody gall-vein enation viruses. Preinoculation of seedlings with a mild strain of tristeza does not interact synergistically with the above pathogens and could, therefore, safely be recommended to overcome the effect of severe strains under normal field conditions.

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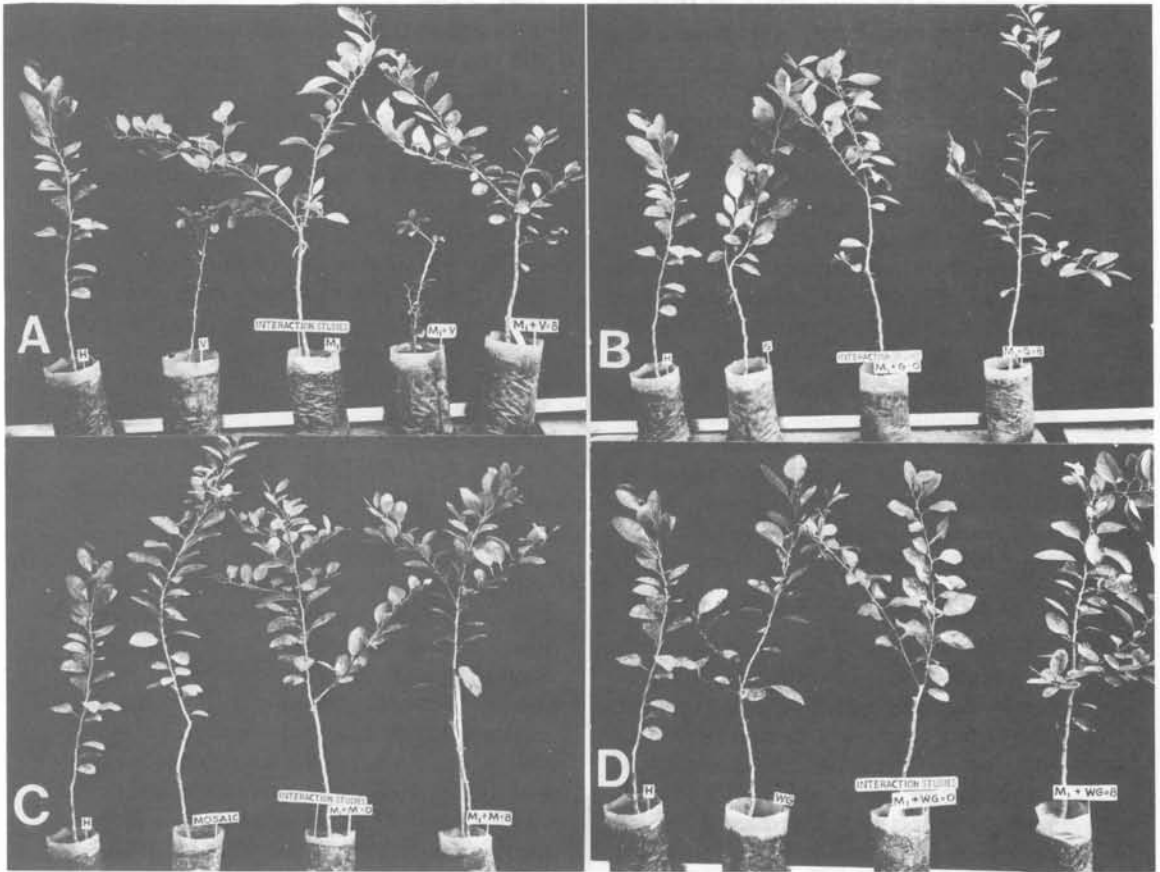


Fig. 1. Interaction between tristeza and other citrus pathogens on acid lime: A) mild and severe tristeza; B) greening; C) mosaic; D) woody gall-vein enation. H = healthy; V = severe tristeza; M<sub>1</sub> = mild tristeza; G = greening, M = mosaic; WG = woody gall-vein enation; M<sub>1</sub> + O = simultaneous inoculation of mild tristeza plus another pathogen; M<sub>1</sub> + 8 = mild tristeza challenge-inoculated with the second pathogen 8 weeks later. Results for infectious variegation, now shown, were similar to mosaic.

TABLE 1  
INTERACTION OF ACID LIME SEEDLINGS INOCULATED WITH A MILD STRAIN OF TRISTEZA VIRUS IN RELATION TO OTHER PATHOGENS

Pathogen	Treatment combinations	Height (cm)				Circumference (mm)			
		Initial	3rd month	6th month	12th month	Initial	3rd month	6th month	12th month
NONE	Healthy	23.0	31.0	55.0	96.7	10.0	19.7	22.6	33.0
TRISTEZA	Mild	22.0	32.3	51.0	99.3	10.0	15.7	21.7	33.7
	Severe	23.0	26.0	27.7	32.0	9.8	10.7	11.0	15.3
	Mild + Severe (0)*	21.0	32.3	35.0	40.0	10.3	11.7	12.0	17.2
	Mild + Severe (8)*	23.3	33.7	48.7	93.0	11.0	16.3	22.0	31.7
	GREENING	Greening	20.7	30.7	49.0	92.0	10.0	15.3	20.3
	Mild† + greening (0)	24.0	33.3	56.0	90.0	10.7	16.7	22.3	32.0
	Mild† + greening (8)	22.7	31.0	51.7	93.0	11.0	17.0	23.3	36.0
INFECTIOUS VARIEGATION (IV)	IV	25.7	30.3	49.3	89.0	9.3	15.0	19.7	30.7
	Mild† + IV (0)	20.7	30.7	54.3	93.0	10.7	15.0	20.0	33.0
MOSAIC	Mild† + IV (8)	22.0	29.3	51.3	94.0	11.0	16.3	22.3	32.0
	Mosaic	24.7	30.3	45.3	97.0	9.7	16.7	21.7	31.3
	Mild† + Mosaic (0)	23.7	29.3	51.7	91.0	10.3	17.7	21.3	34.0
WOODY GALL — VEIN ENATION (WG)	Mild† + Mosaic (8)	24.7	28.7	53.7	89.0	10.0	22.0	24.7	35.3
	WG	22.0	29.0	37.0	98.7	9.0	16.0	20.7	30.3
	Mild† + WG (0)	22.3	31.0	53.7	93.7	10.0	16.0	21.0	33.3
	Mild† + WG (8)	22.3	29.3	51.3	97.0	10.0	18.0	22.0	31.3
	SE <sub>1/2</sub> ‡	N.S.	N.S.	2.5	5.3	N.S.	2.6	1.5	2.7
	C.D.			7.9	10.8		5.3	3.6	5.7

\* 0 = simultaneous inoculation; 8 = inoculation of severe strain 8 weeks later.

† Mild tristeza.

‡ Significant at 1% level.

TABLE 2  
TRISTEEZA SYMPTOMS, ROOT AND SHOOT WEIGHTS OF ACID LIME 1 YEAR  
AFTER INOCULATION WITH TRISTEEZA VIRUS AND OTHER PATHOGENS

Pathogen	Treatment Combinations	Vein flecks/ leaf	Stem pits/ 10 cm stem	Root wt g	Shoot wt. g	Girth mm
NONE	Healthy	0.0	0.0	28.0	70.5	11.5
TRISTEEZA	Mild	3.6	2.9	34.5	66.5	11.0
	Severe	13.4	10.2	12.5	16.5	5.5
	Mild + severe (0)*	12.8	9.5	9.5	17.5	5.0
	Mild + severe (8)*	3.3	3.1	29.3	75.0	10.5
	GREENING	Greening	0.0	0.0	29.0	68.0
GREENING	Mild† + greening (0)	3.1	2.6	33.0	69.0	12.5
	Mild† + greening (8)	2.9	3.0	30.0	75.5	13.5
	INFECTIOUS	IV	0.0	0.0	29.5	63.0
VARIEGATION (IV)	Mild† + IV (0)	3.5	3.2	32.5	66.5	11.5
	Mild† + IV (8)	3.1	2.9	37.0	67.0	12.5
MOSAIC	Mosaic	0.0	0.0	32.0	67.5	11.0
	Mild† + mosaic (0)	2.9	2.8	27.5	65.0	11.0
	Mild† + mosaic (8)	3.0	3.1	28.0	62.5	12.0
WOODY GALL — AND VEIN ENATION (WG)	WG	0.0	0.0	28.5	75.0	11.0
	Mild† + WG (0)	3.5	2.7	34.5	72.5	11.0
	Mild† + WG (8)	3.7	3.3	32.0	65.0	12.0
	SE <sub>df</sub> ‡	3.0	8.7	0.43	0.36	1.36
	CD	6.8	17.5	0.81	0.78	2.74

\* 0 = simultaneous inoculation; 8 = inoculation of severe strain after 8 weeks.

† Mild tristeza

‡ Significant at the 1% level; mean of 3 replications.

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