Evidences that Tristeza and Stem Pitting are Different Viruses or Components of the Same Complex

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ABSTRACT. Results indicated that tristeza and stem pitting are diseases caused by different viruses or components of the tristeza complex. Five-year-old, Satsuma mandarin trees growing vigorously on sour orange rootstock were found to be infected with severe tristeza stem pitting virus. The same scion budded on Morton citrange and on *Citrus taiwanica* produced trees which showed mild to very severe wood pitting on the rootstock. Superinoculation of Satsuma trees on sour orange with severe tristeza, by the use of *Toxoptera citricida* Kirk and by tissue grafting, failed to induce tristeza symptoms. Young plants of Valencia and Pera sweet oranges on sour orange rootstock, aphid inoculated with the stem pitting virus are healthy, while inoculated Valencia plants on Mexican lime rootstock are declining. Mexican lime seedlings reacted to the stem pitting virus in Satsuma with severe leaf cupping and yellowing, vein corking, stunting, extreme wood pitting and sometimes honeycombing.
Index words. Sour orange, Mexican lime, seedling yellows.

Citrus tristeza virus (CTV) is considered the causal agent of three types of diseases or plant reactions, namely: stem pitting, seedling yellows and tristeza itself. It is not as a simple entity, but is as a complex of strains, ranging from mild to very severe. Indexing for CTV is normally by inoculation to small acid limes (Mexican, Key, West Indian, Beledy, Galego) which are considered nearly infallible indicators for detecting the presence of the virus. All strains of the tristeza virus, even the very mild, are detected by vein clearing in the young leaves and pit lesions in the vascular tissues of the lime plants. Supplementary indicators, like sour orange for seedling yellows and composite trees of sweet orange scion on sour orange rootstock for tristeza are less frequently used. Maybe, for this reason the possibility that tristeza and stem pitting are caused by different viruses or strains of the complex has been overlooked.

Wallace (10), Fraser (4) and McClean (5) have pointed out the possibility of tristeza and seedling yellows being two distinct viruses. However, so far, all sources of the virus that induce seedling-yellows also cause a stem pitting reaction in lime seedlings and tristeza symptoms in trees of sweet orange on sour orange rootstock. Many authors (5, 7, 8, 9) on the other hand, have considered stem pitting a symptom of tristeza virus or of tristeza strains, pointing out that stem pitting may cause more economic losses than the tristeza effect itself.

EXPERIMENTS AND RESULTS

Source of stem pitting virus. A germplasm bank of citrus varieties was established at the S. Manoel Experiment Station, UNESP, Botucatu, starting in 1973. Nine trees of nucellar Satsuma mandarin budded on Volkamer lemon were included in this plot. They were produced with budwood brought from the Limeira Experiment Station, from three different nucellar seedlings grown from seeds planted in 1955.

The most vigorous and productive of those Satsuma mandarin trees was used as a mother tree for a rootstock experiment, including 13 rootstock varieties. Seeds of the rootstocks were planted in April 1977. All budding with

Satsuma mandarin was done in January 1978 and the trees were transplanted to the field on October 31, 1980. Some tristeza intolerant rootstocks were included in the experiment. In the nursery, 30 seedlings of each rootstock were budded with the nucellar Satsuma mandarin scion and another 30 seedlings with nucellar Pera orange, for another similar rootstock experiment.

In the basic nursery all Pera orange scions on sour orange and on other tristeza intolerant rootstocks died within 10 months, following initial healthy growth. All Satsuma scions grew well.

A Satsuma mandarin rootstock experiment was established at the "Presidente Medici" Experiment Station, UNESP, Botucatu. A randomized block design was used, with two-tree plots and four replications. On May 1983, all trees on sour orange and Red shaddock were still growing vigorously and producing the first fruits (table 1). Trees on Morton citrange were stunted, and exhibited severe wood pitting in the trunk below the bud-union. The trees on *Citrus taiwanica* (selection from Florida) also showed wood pitting in the rootstock portion of the trunk, but to a lesser degree.

Nine Satsuma mandarin trees at the S. Manoel Experiment Station revealed some conoid wood pitting in the trunk above the bud-union in all trees. In two cases the pitting descended to the rootstock.

Tristeza inoculation tests. Twenty Satsuma mandarin scions on sour orange rootstock, that remained in the nursery were selected for a tristeza inoculation test. Ten plants were inoculated with buds of Pera orange known to be carrying a severe strain of tristeza virus and another ten plants were left as non-inoculated controls. Four of the eight Satsuma trees on sour orange in the experiment were also inoculated with buds from the same source.

Inoculations were made on April 1982 and one year later all

TABLE 1
AVERAGE TREE HEIGHT AND FRUIT PRODUCTION IN 1983 SEASON* OF SATSUMA MANDARIN BUDDED ON 13 ROOTSTOCKS

Rootstock	Average tree height (cm)	Average fruit production per tree	Comments
Volkamer lemon	188	170	Vigorous plants, very poor quality fruits
Sunki mandarin	185	08	
Cleopatra mandarin	176	00	
Citrus taiwanica	170	03	Mild wood pitting below bud union
Sour orange	169	14	healthy plants
Florida rough lemon	168	90	
Rangpur lime	151	32	
Red shaddock	145	01	healthy plants
Brazilian rough lemon	130	25	
Caipira sweet orange	124	01	
Trifoliate orange	122	01	
Morton citrange	121	78	Severe wood pitting
Carrizo citrange	113	06	below bud union

*Trees planted in the field on 31 October 1980. Readings on 14 December 1982.

plants remained alive without any tristeza symptoms. Severe infestation of the black aphid, *Toxoptera citricidus* Kirk., occurred in the test plants and neighbouring citrus orchards, and this should have resulted in movement of tristeza virus throughout the experimental plants.

To explain this result, it is proposed that the Satsuma mandarin was carrying a stem pitting virus or a strain of tristeza that injured certain citrus types but caused no damage to sour orange. This virus or strain of tristeza named CTV-SP (citrus tristeza virus - stem pitting) somehow protected the Satsuma trees from further superinoculation with severe tristeza strains.

Indexing trials. Eighteen young Mexican lime seedlings growing in pots under greenhouse conditions were inoculated with buds taken from one of the healthy Satsuma mandarin trees on sour orange rootstock. Each seedling was inoculated with a single blind bud on April 1982. Three seedlings were inoculated with buds from a Pera orange trees carrying severe tristeza virus and another three plants were left non-inoculated, to serve as positive and negative tristeza control plants. Monthly inspections were made on all test plants. All inoculated seedlings developed tristeza symptoms that ranged from mild to very severe.

These results indicated that the Satsuma tree on sour orange rootstock growing without symptoms in the field, was carrying a virus that induced a tristeza reaction in the Mexican lime test plants.

Another experiment was conducted, using *Toxoptera citricidus* Kirk as vector of tristeza virus, from field trees into young Mexican lime seedlings in the screenhouse. Aphids were collected on August 1982 from Satsuma mandarin trees thought to be carrying CTV-SP and from Hamlin orange trees known to be carrying CTV-SY (Citrus tristeza virus-seedling yellows) and transferred to the Mexican lime seedlings. A minimum of 20 adult aphids were allowed to feed for 24 hours on the test plants. Eight plants were inoculated with CTV-SP and 8 plants with CTV-SY plus 8 healthy control plants. Aphids were killed with malatol spray and the plants returned to the greenhouse. In November 1982, 15 of the 16 aphid inoculated seedlings were showing severe reaction, independent of the plant from which the insects were collected. One Mexican lime seedling inoculated with CTV-SP showed mild tristeza symptoms. Symptoms of severe reaction included stunting, yellowing of leaves, severe vein corking and intense stem pitting.

It was then postulated that the tissues of Satsuma mandarin was preventing the multiplication of the CTV-SY component and allowing the proliferation of the stem pitting virus. Another experiment did not confirm this assumption and indicated that the Satsuma plants were carrying CTV-SP or a stem pitting virus.

Grown citrus aphids were used to inoculate young healthy scions of nucellar Valencia and Pera oranges and Satsuma mandarin on sour orange rootstock and nucellar Valencia orange on Mexican lime rootstock with CTV-SP. Mexican lime seedlings were similarly infected in this trial. Six test plants of each kind were inoculated and another six plants were kept as healthy controls. Inoculations were made on August 1982. Nine months later all scions on sour orange rootstock were growing without symptoms. The Valencia orange scions on Mexican lime rootstock were stunted with small yellow leaves, suggestive of tristeza symptom. All inoculated Mexican lime seedlings were stunted, growth had stopped,

TABLE 2
AVERAGE HEIGHT AND ASPECT OF PLANTS, NINE MONTHS AFTER APHID INOCULATION WITH STEM PITTING VIRUS (CTV-SP)*

Plant	Inoculated		Uninoculated	
	height (cm)	appearance	height (cm)	Reduction (%)
Valencia/Sour	25.83	healthy	26.33	1.90
Pera/Sour	21.33	healthy	20.00	—
Satsuma/Sour	23.00	healthy	18.67	—
Valencia/Mexican lime	13.33	Stunted, yellowing	16.67	20.04
Mexican lime seedlings	18.83	Severe vein corking	37.83	50.22

*Aphid inoculation on August 1982 and final readings on May 1983.

and leaves showed cupping, yellowing and extremely severe vein corking. No indication of the presence of citrus vein enation virus was found in these experiments.

Further experiments, including superinoculation of the orange trees on sour orange rootstock with CTV-SY are underway.

DISCUSSION AND CONCLUSIONS

All citrus trees comprised of sweet oranges, mandarins and grapefruit scions budded on sour orange were destroyed in Brazil, following the invasion of tristeza virus disease, which started in 1937. The only surviving tree, the famous Catu sour orange was found to be a sour hybrid, and is partially tolerant to the virus. Tristeza became endemic in practically all citrus trees (except for true lemons and a few other citrus types) and further attempts to use sour orange as rootstock resulted in complete failure.

It was, therefore, unexpected to find Satsuma mandarin trees budded on sour orange, making vigorous growth, even though they were infected with a severe stem pitting tristeza-like virus. This is occurring in one area where other citrus trees are carrying severe CTV-SY and where continuous movement of the brown citrus aphid occurs.

Florida is the only area where a large number of citrus trees on sour orange rootstock are known to be carrying CTV and surviving (1, 2, 3, 6). Where this happens, explanations like the occurrence of extremely mild strains of tristeza, cross protection or partially tolerant types of sour orange are suggested.

If CTV and CTV-SP are different viruses or components of the tristeza complex, as suggested by this study, this would suggest a further explanation for the existence of healthy looking tristeza infected trees on sour orange rootstock.

The sour orange selection used in the present work is highly intolerant to CTV, as found by the quick decline with Pera orange scions.

The superinoculation tests indicated that the CTV-SP virus was given protection against severe CTV-SY. The healthy growth of inoculated sour orange seedlings indicate that CTV-SP is not carrying the seedling yellows component.

McClean (5) has reported that severe strains of tristeza, as measured by the behaviour of lime seedlings are not necessarily able to induce tristeza disease. However,

this author did not discuss the possibility of using such CTV strains to protect sweet orange trees on sour orange rootstock from further infection with CTV-SY strains.

Additional research work is underway, but strong evidence was obtained that CTV and CTV-SP are different components of the tristeza complex.

LITERATURE CITED

1. BRIDGES, G. D. and C. O. YOUTSEY
   1972. Natural tristeza infection of citrus species, relatives and hybrids at one Florida location from 1961-1971. Proc. Florida State Hort. Soc. 85: 44-47.
2. COHEN, M.
   1956. Incidence of tristeza virus in Florida in trees not yet showing field symptoms. Phytopathology 46: 9.
3. COHEN, M.
   1976. A comparison of some tristeza isolates and a cross-protection trial in Florida. p. 50-54. *In* Proc. 7th Conference IOCV. Univ. California, Riverside.
4. FRASER, L.
   1958. Virus diseases of citrus in Australia. Proc. Linnean Soc. N.S.W. 73: 9-19.
5. MCCLEAN, A. P. D.
   1960. Seedling-yellows in South African citrus trees. South African J. Agr. Sci. 3(2): 259-279.
6. NORMAN, G., W. C. PRICE, T. J. GRANT, and H. BURNETT
   1961. Ten years of tristeza in Florida. Proc. Florida State Hort. Soc. 74: 107-111.
7. ROISTACHER, C. N.
   1982. The destructive potential for seedling yellows. Citrograph 67(3): 48-53.
8. SALIBE, A. A.
   1973. The tristeza disease. p. 68-76. Proc. Intern. Citrus Short Course. Univ. Florida, Gainesville.
9. SALIBE, A. A.
   1977. The stem-pitting effects of tristeza on different citrus hosts and their economic significance. 1977 Proc. Intern. Soc. Citriculture 3: 953-955.
10. WALLACE, J. M.
   1957. Tristeza and seedling yellows of citrus. Plant Dis. Reptr. 41: 394-397.