Proc. Fla. State Hort. Soc. 103:83-87. 1990.

SEEDLING YELLOWS ISOLATES OF CITRUS TRISTEZA VIRUS IN COMMERCIAL CITRUS IN FLORIDA

S. M. GARNSEY
U.S. Horticultural Research Laboratory
USDA/ARS, 2120 Camden Road
Orlando, FL 32803

Abstract. **The seedling yellows (SY) reaction is considered indicative of severe strains of citrus tristeza virus (CTV). Historically, SY has been rare in Florida, except in some imported cultivars such as Meyer lemon (*Citrus limon* L. [Burm. f.] hybrid?). Recently, over 75 isolates of CTV, which included standard isolates, isolates taken from declining mature trees on sour orange (*C. aurantium* L.) rootstock, isolates from stunted young trees on sour orange rootstock and isolates from symptomless trees on sour orange and/or CTV-tolerant rootstocks were indexed for SY on sour orange seedlings. Thirty-three isolates induced mild to strong SY. These were found in field trees, in stunted young trees in commercial plantings, and in registered budwood source trees. SY isolates were recovered from multiple locations and are apparently being spread in Florida by aphid vectors in mature plantings and by propagation of SY-infected budwood. SY was not induced by mild isolates of CTV. Some decline or stunt-inducing isolates also did not cause SY.**

Seedling yellows (SY) is a term used to describe the reaction caused by some isolates of citrus tristeza virus (CTV) in inoculated seedlings of sour orange, acid lemons (*C. limon* [L.] Burm. f.) and grapefruit (*C. paradisi* Macf.). SY is not a field disease, but rather an indicator plant reaction which is indicative of a severe strain of CTV (10,15). SY isolates of CTV normally cause a strong decline reaction in sweet orange (*C. sinensis* [L.] Osbeck), mandarin (*C. reticulata* Blanco), or grapefruit trees budded on sour orange rootstock. SY isolates also frequently cause stem pitting in grapefruit or sweet orange scions regardless of rootstock, but some severe stem pitting isolates do not cause SY (3). Some decline-inducing isolates of CTV also do not cause SY (6,9,15). As in the case of other CTV-induced symptoms in citrus, the degree of SY reaction can vary from mild to severe according to the isolate involved. Mild SY reactions may be difficult to recognize unless test conditions are ideal.

SY isolates of CTV historically have been rare in Florida, and SY has been reported primarily from citrus varieties introduced from areas where SY-CTV is common (4). The most common example is 'Meyer' lemon (*C. limon* hybrid?) which was introduced into the U.S. in 1908 from China and into Florida prior to 1920 (4). Natural spread of CTV from 'Meyer' lemon apparently is not common in either California or Florida (4,13). SY isolates of CTV were undoubtedly introduced into Florida prior to 1900 with movement of satsuma (*C. unshui* [Mack.] Marc.) mandarin trees from Japan (16). This assumption is based on the reports that all attempts to propagate satsuma on sour orange failed completely (16) and that SY isolates are common in mandarins from Japan (14,15). There is little evidence that CTV spread from these trees before they were eliminated by freezes or replaced by other varieties.

Recently, CTV decline has become an increasing problem in mature trees on sour orange, especially in South Florida (2). Stunting of young trees budded on sour orange in the nursery or in young field plantings has also become widespread and caused major losses (2). In the past, nurserymen have successfully propagated budwood infected with CTV on sour orange and produced vigorous trees. These observations have raised questions about a change in severity of CTV isolates in Florida. Lee and Brlansky (9) recently tested 25 CTV isolates from trees in various stages of decline and did not find any that caused SY. However, biological characterization of the T36 isolate of CTV, which has been used extensively in experimental studies of CTV and for production of monoclonal and polyclonal antibodies (11,12), indicated that it produced a moderate SY response. This isolate was isolated originally from a quick decline tree in Winter Garden (7,17).

This paper presents results of further tests of Florida CTV isolates and confirms that SY isolates of CTV are present in field trees in various areas of Florida. Implications of these results for future citrus production are discussed.

Materials and Methods

Virus isolates. Several standard isolates of CTV from Florida were included in these tests (Table 1). Most of these have been previously described. T3 is a severe source isolated in Florida by T. J. Grant (8). It probably originated from an introduced citrus selection. T36 is an aphid-transmitted isolate of CTV which was obtained from a quick decline field source. Two mild variants of T36 and T3 were also included. These isolates were obtained in previous studies by repeated mechanical transmission of partially purified preparations (5), and produce much milder symptoms in Mexican lime (*C. aurantifolia* [Christm.] Swing.) than the parent isolates. The T36 variant tested produces only very mild vein clearing in Mexican lime. T68 is a CTV source which was originally isolated from an 'Ellendale' mandarin tree propagated from budwood imported into Florida without authorization. The field source was destroyed after its discovery, but a glasshouse propagation of the virus has been maintained for reference purposes. Seven mild isolates of CTV which have been used in cross-protection studies (18) were also tested.

The remaining virus isolates tested in this study were obtained from field or nursery trees growing in different parts of Florida. Many of the isolates tested were from mature trees on sour orange rootstock with severe decline symptoms. These were collected from areas with outbreaks of quick decline in central and south Florida (2,7). Some isolates were obtained from severely stunted young trees on sour orange in nursery or young field plantings, or from budwood sources that had produced stunted trees when propagated on sour orange (S. M. Garnsey, et al., unpublished; C. O. Youtsey, personal communication). Twig or leaf tissues from these trees were used to inoculate

Florida Agricultural Experiment Station Journal Series No. 0-000.

healthy Mexican lime or sweet orange plants in the greenhouse to establish experimental sources of each isolate. The isolates were coded with a FS (field sample) or T (tristeza) number. Some of the isolates from older field trees were undoubtedly infected with other virus or viroid pathogens, but a complete indexing for these was not done. Isolates from naturally infected nucellar lines were presumed free of other virus and viruslike pathogens. It is also presumed that many of the declining field trees had been infected with a mild isolate of CTV for a number of years without significant effect and then subsequently aphid-inoculated with a decline-inducing isolate of CTV. These sources probably contain a mixture of isolates.

Indexing for Seedling Yellows. Vigorous glasshouse-grown sour orange seedlings were grown in a steam-sterilized potting medium in 12.5- or 15-cm plastic pots. Plants were inoculated when stem diameter reached 3-4 mm and plant height was 30 to 45 cm. Plants were graft-inoculated using 2 blind buds, side grafts or leaf pieces per plant. Plants were cut back either immediately after inoculation, or when the grafting tape was removed and inoculum viability was verified 2 weeks after inoculation. Symptoms were read as flushes of new growth were approaching maturity. Several readings were made, but primary emphasis was placed on the second flush after inoculation. In some cases, plants were cut back again to promote uniform secondary flushes.

Double sandwich enzyme linked immunoabsorbent assay (ELISA) (1) was used to verify CTV infection in indicator plants which were inoculated but showed no definite SY symptoms. Young flush tissue was harvested from inoculated plants at the termination of the experiment, ground in PBS-T extraction buffer (1) at a 1/20 dilution and tested with the polyclonal antiserum 1052 to the T36 isolate of CTV. Plates were read with a Biotek 309 plate reader zeroed to buffer in a blank well. Samples were considered positive when the OD_{405} value was twice that of extracts from healthy sour orange or the value for the healthy extract value-plus 0.10 if the OD_{405} was less than 0.05.

Symptoms were evaluated on a scale of zero to three, where zero = no visual differences from the uninoculated controls; one = a definite yellowing of the new flush relative to uninoculated controls and slight stunting or leaf size reduction; two = a strong yellowing in the new flush, a marked reduction in leaf size, and a moderate stunting of shoot growth; and three = severe yellowing and size reduction of new flush leaves and severe stunting. An overall rating for the isolate was obtained by averaging the readings for the five individual indicators.

Results

Typical SY symptoms were produced in sour orange indicators by known SY sources under our glasshouse conditions. Symptoms were clear, even in summer, when day temperatures were somewhat higher than ideal. Typical symptoms are shown in Fig. 1 and 2.

Several standard sources of CTV from Florida were tested along with the field sources evaluated. The results for these standard sources are presented in Table 1 along with a summary of their other biological properties. Three isolates which had produced strong SY in previous tests (T3, T36, and T68) produced good SY reactions. Seven

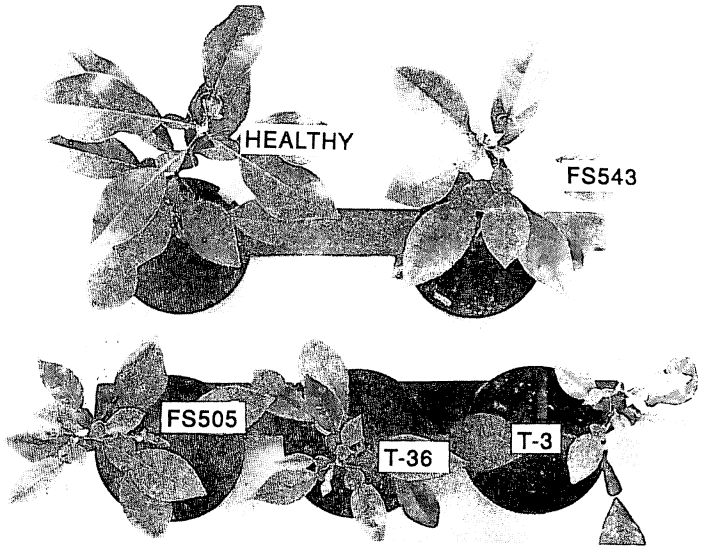

Fig. 1. Varying intensity of seedling yellows (SY) symptoms in young sour orange seedlings inoculated with different sources of citrus tristeza virus. FS543 and FS505 originally were obtained from stunted young trees. T36 is from a quick decline source, and T3 is a standard source of severe SY. Both leaf size and color are affected.

mild sources of CTV which do not produce decline in trees on sour orange rootstock and have been used experimentally for mild cross protection did not cause SY. In addition to the results shown in Table 1, two isolates of CTV from 'Meyer' lemon positive for SY in previous tests (4) also produced SY in these tests. The variants of T3 and T36, selected on the basis of milder symptoms in Mexican lime, produced strong SY symptoms which were slightly milder than those produced by the parent isolate.

Twenty-eight isolates were tested which originally had been isolated from mature field trees showing CTV decline symptoms. As shown in Table 2, 21 of the 28 isolates tested caused some SY response, and 16 of these were moderate to strong. The SY response was not confined to isolates from a single location. Six isolates from apparently vigorous trees in areas where quick decline was rampant were negative for SY.

Twenty-one isolates were tested which had been collected directly from stunted young trees on sour orange in the field, or the nursery or from budwood source trees which had yielded stunted progeny when propagated on sour orange. As shown in Table 2, 19 isolates produced a noticeable SY response and six of these produced rather strong symptoms. Two isolates of CTV from vigorous, young trees in plantings where stunting was prevalent did not produce SY.

Although this study focused primarily on CTV sources from sour orange-rooted trees, several isolates were checked from trees on CTV-tolerant rootstocks. SY was also obtained from a normal-looking 'Temple' tree (*C. sinesis* hybrid) near Dundee and from a stunted young nursery tree on Carrizo citrange (*C. sinensis* X *Poncirus trifoliata* [L.] Raf.).

The origin of 12 of the most severe SY sources is given in Table 3. It can be seen that SY is not unique to a single location or tree age and that the symptom noted in Florida commercial sources are comparable to those from the two 'Meyer' lemon sources.

Fig. 2. Comparative reaction of three Florida CTV sources in graft-inoculated seedlings of sour orange. (A) Healthy control, (B) inoculated with FS447 (decline isolate, Winter Garden), (C) inoculated with FS547 (stunted young tree, Dundee), (D) inoculated with FS406 (decline isolate, Dundee), and (E) inoculated with T3 (the SY positive control).

Discussion

Clearly, isolates of CTV from Florida can cause SY. It has been known that some exotic sources of SY existed, but presence of SY in commercial sources has not been reported. The indexing for SY is not especially easy to evaluate because the stunting and chlorosis symptoms are not highly specific and can be confused with other horticultural or disease problems. Mild SY symptoms are, in fact, probably overlooked or considered inconclusive in many cases. Vigorous, healthy growth in control seedlings is essential for reference purposes, especially to detect mild forms of SY. The SY symptoms obtained from many sources reported here, however, were clear and reproducible. No field sources were found which caused symptoms as severe as those caused by T3 and T68, but there were sources comparable to those produced by two 'Meyer' lemon isolates. Some sources gave very mild reactions, but these again were reproducible. Not all sources of CTV which cause decline in sweet/sour trees will cause SY, and that was also evident in these tests. All of the SY sources identified here which have been well indexed in sweet/sour plants have produced stunting or decline reactions.

Table 1. Seedling yellows (SY) response in sour orange seedlings inoculated with standard Florida isolates of citrus tristeza virus (CTV).

Code	Indicator reaction			Current SY rating
	Mex li[z]	Swt/Sour[y]	Sour[x]	
T3	Strong	Strong	Strong	2.5
T36	Strong	Mod.	Mod.	2.0
T68	Strong	Strong	Strong	2.5
T4	Mod.	Neg.	Neg.	0
T11	Mild	Neg.	Neg.	0
T26	Mild	Neg.	Neg.	0
T28a	Mild	Neg.	Neg.	0
T30	Mild	Neg.	Neg.	0
T55a	Mild	Neg.	Neg.	0
T69	Mild	Neg.	Neg.	0
Healthy	None	None	None	0

[z]Vein clearing, stem pitting, and stunting in Mexican lime.
[y]Stunting and chlorosis in young sweet orange grafted on sour orange.
[x]Stunting and chlorosis in sour orange seedlings.

The two SY isolates from 'Meyer' lemon, T68, and probably T3, are traceable to exotic varieties already infected with SY when introduced. However, SY isolates found in declining mature trees in commercial groves were almost certainly introduced into these trees in the field by aphid vectors. An alternate explanation that mutation of existing mild isolates to a SY form is occurring simultaneously in thousands of field trees in different locations is unlikely. Aphid transmission of SY isolates from Florida has been demonstrated (17), and, in fact, the T36 isolate used as a reference in these tests is an aphid-transmitted subculture from a quick decline field source.

The SY isolates in stunted young field trees presumably represent propagation of SY from budwood source trees carrying SY rather than natural spread in nursery or field sites. However, introduction of SY into the parent scion mother trees presumably occurred by aphid inoculation since many of these were originally CTV-free.

The extent of SY movement by budwood propagation or the distribution of SY isolates in trees on CTV-tolerant rootstock was not studied. However, some of the same budwood sources that indexed positive for SY and cause stunting when propagated on sour orange have been extensively propagated on other rootstocks and planted commercially. These SY isolates apparently do not cause severe stem pitting symptoms in grapefruit or sweet orange and

Table 2. Seedling yellows (SY) response in sour orange seedlings inoculated with field sources of citrus tristeza virus from Florida[z].

Tree age	Condition	Location		No. of trees	No. plants in each category[y] 0	<0.5	0.5-1.5	1.5-2.5	>2.5
Mature	Decline	Polk/Dundee		6	0	3	3	0	0
Mature	Decline	Winter Garden		9	2	0	4	3	0
Mature	Decline	Southeast		5	1	2	2	0	0
Mature	Decline	Southwest		3	1	0	1	1	0
Mature	Decline	Misc.		5	3	0	2	0	0
			Subtotal	28	7	5	12	4	0
Young	Stunted	Polk/Dundee		15	2	2	7	4	0
Young	Stunted	Southwest		6	0	2	2	2	0
			Subtotal	21	2	4	9	6	0
			TOTAL	49	9	9	21	10	0
Mature	Healthy			6	6	0	0	0	0
Young	Healthy			2	2	0	0	0	0
			TOTAL	8	8	0	0	0	0

[z]All sources from sweet orange or grapefruit trees grafted on sour orange rootstock or from budwood sources which have yielded stunted trees when propagated on sour orange rootstock.
[y]SY rated on a scale of 0 (no symptoms) to 3 (severe leaf chlorosis and stunting).

do not cause obvious effects in tolerant scion/stock combinations. These Typhoid Marys may, however, have undetected reduction in yield and vigor and greatly increase the inoculum reservoir of CTV which can affect existing plantings on sour orange. Since these trees do not decline, the reservoir of SY is maintained.

All of the SY isolates reported here can be easily distinguished from mild, nondecline isolates by a rapid serological assay (11,12). It is not feasible to identify and destroy all SY-infected sources in commercial plantings in Florida. It is feasible, however, to prevent further spread of SY isolates by propagation. Budwood sources could be quickly and easily tested, and those that test positive for SY or decline isolates should be avoided.

The presence of SY is considered a danger signal for evaluating CTV effects (14,15). Isolates that give a SY response are expected to cause decline in sour orange-rooted trees and frequently stem pitting in commercial scions. As such, SY is a valuable indicator, even the SY reaction however is not a completely accurate barometer for severe CTV, since some isolates of CTV in other countries which cause severe stem pitting in grapefruit or sweet orange either do not cause SY, or only a very mild reaction (3).

Table 3. Origin of some Florida citrus tristeza virus (CTV) isolates that cause a moderate to strong seedling yellows (SY) response in sour orange seedlings.

Isolate	Description	SY rating[y]
FS43	Meyer lemon, Winter Haven	2.0
FS84	Meyer lemon, Ruskin	1.5
FS217	Quick Decline, Winter Garden	2.0
FS319	Quick Decline, Winter Garden	2.0
FS323	Quick Decline, Clermont	1.5
FS451	Stunting Source[z], Avon Park	1.5
FS492	Quick Decline, Hendry Co.	1.5
FS505	Stunting source, Hendry Co.	1.5
FS528	Stunting source, Hendry Co.	1.5
FS541	Stunting source, Dundee	1.5
FS544	Stunting source, Dundee	1.5
FS550	Stunting source, Dundee	1.5

[z]Stunting sources were either stunted young trees propagated on sour orange, or trees known to produce stunted nursery trees when propagated on sour.
[y]Seedling yellows rated on scale of 0 (no symptoms) to 3 (very severe stunting and chlorosis).

Acknowledgments

The expert technical assistance of Carmen Henderson, Joyce Bittle, and Randy Smith is gratefully acknowledged. Charles Youtsey also generously provided CTV isolates for testing and information on the properties of certain isolates.

Literature Cited

1. Bar Joseph, M., S. M. Garnsey, D. Gonsalves, M. Moscovitz, D. E. Purcifull, M. F. Clark, and G. Loebenstein. 1979. The use of enzyme linked immunosorbent assay for detection of citrus tristeza virus. Phyotopathology 69:190-194.
2. Brlansky, R. H., R. R. Pelosi, S. M. Garnsey, C. O. Youtsey, R. F. Lee, R. K. Yokomi, and R. M. Sonoda. 1986. Tristeza quick decline epidemic in south Florida. Proc. Fla. State Hort. Soc. 99:66-69.
3. Da Graca, J. V., L. J. Marais, and L. A. Von Broembesen. 1984. Severe tristeza stem pitting decline of young grapefruit in South Africa. p. 62-65, *In* S. M. Garnsey et al. (eds.) Proc. 9th Conf. Intern. Organ. Citrus Virol., IOCV, Riverside, CA.
4. Garnsey, S. M. 1970. Viruses in Florida's 'Meyer' lemon trees and their effects on other citrus. Proc. Fla. State Hort. Soc. 83:66-71.
5. Garnsey, S. M. 1984. Separation of citrus tristeza virus strains and strain variants by stem-slash inoculation of citrus receptors. Phyotopathology 74:848.
6. Garnsey, S. M., E. L. Civerolo, D. J. Gumpf, R. K. Yokomi, and R. F. Lee. 1991. Development of a worldwide collection of citrus tristeza virus isolates. *In* R. H. Brlansky et al. (eds.) Proc. 11th Conf. Intern. Organ. Citrus Virol., IOCV, Riverside, CA. (In press)
7. Garnsey, S. M. and J. L. Jackson. 1975. A destructive outbreak of tristeza in central Florida. Proc. Fla. State Hort. Soc. 88:65-69.
8. Grant, T. J. and R. P. Higgins. 1957. Occurrence of mixtures of tristeza virus strains in citrus. Phytopathology 47:272-276.

9. Lee, R. F. and R. H. Brlansky. 1989. Variation in the severity of citrus tristeza virus isolates from groves with quick decline. Proc. Fla. State Hort. Soc. 102:1-3.
10. McClean, A.P.D. 1974. The tristeza virus complex. p. 59-66. In: L. G. Weathers, and M. Cohen (eds.). Proc. 6th Conf. Intern. Organ. Citrus Virol., Univ. of Calif., Div. Agric. Sci., Richmond.
11. Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee. 1991. A monoclonal antibody that discriminates strains of citrus tristeza virus. Phytopathology 80:224-228.
12. Permar, T. A. and S. M. Garnsey. 1991. Comparison of biological indexing and immunological assays for identifying severe Florida isolates of citrus tristeza virus. *In* R. H. Brlansky et al. (eds.) Proc. 11th Conf. Intern. Organ. Citrus Virol., IOCV, Riverside, CA. (In press)
13. Roistacher, C. N. 1981. Blueprint for disaster—Part two: Changes in transmissibility of seedling yellows. Citrograph 67:28-32.
14. Roistacher, C. N. 1982. Blueprint for disaster—Part Three: The destructive potential for seedling yellows. Citrograph 67:48-53.
15. Roistacher, C. N. and D. J. Gumpf. 1986. A historical perspective on seedling yellows virus. Citrograph 71:189-192.
16. Swingle, W. T. 1909. The limitation of the satsuma orange to trifoliate-orange stock. Circ. No. 46, U.S. Dept. of Agric., Bureau of Plant Industry, Wash. DC.
17. Yokomi, R. K. and S. M. Garnsey. 1987. Transmission of citrus tristeza virus by *Aphis gossypii* and *Aphis citricola* in Florida. Phytophylactica 19:169-172.
18. Yokomi, R. K., S. M. Garnsey, T. A. Permar, R. F. Lee, and C. O. Youtsey. 1991. Natural spread of severe citrus tristeza virus isolates in citrus preinfected with mild CTV isolates. *In* R. H. Brlansky et al. (eds.) Proc. 11th Conf. Intern. Organ. Citrus Virol., IOCV, Riverside, CA. (In press)