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A SURVEY FOR CITRUS TRISTEZA VIRUS IN REGISTERED BUDWOOD SOURCES COMMERCIALLY PROPAGATED ON SOUR ORANGE ROOTSTOCKS IN FLORIDA[1]

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Abstract. **In the year ending June 30, 1979, over 525,000 registered nursery propagations on sour orange** (Citrus aurantium **L.) rootstocks were reported to the Citrus Budwood Registration Bureau. The buds came from 291 registered trees in 9 locations. We indexed 260 of these trees for citrus tristeza virus (CTV) by enzyme-linked immunosorbent assay (ELISA), a rapid, sensitive serological procedure. Selected trees were also indexed by graft-inoculation of 'Mexican' lime** (C. aurantiifolia **(Christm.) Swing.) seedlings and/or by serologically specific electron microscopy. Three of 32 grapefruit** (C. paradisi **Macf.) budwood-source trees and 199 of 228 sweet orange** (C. sinensis **(L.) Osb.), 'Temple' orange** (C. sinensis **hybrid), and tangelo** (C. paradisi **X** C. reticulata **Blanco) trees were infected. Because CTV is readily bud-transmitted from infected trees, we estimate that approximately 380,000 CTV-infected, registered sweet orange trees were propagated commercially on sour orange rootstock in the 1978-79 period. Results obtained by ELISA and by indexing on 'Mexican' lime were closely correlated. The effects of this CTV infection are under further investigation.**

Introduction

Citrus tristeza virus (CTV) has become widespread throughout Florida since its discovery here almost 30 years ago (12). Severe CTV-induced decline has occurred in citrus trees on sour orange rootstocks in several areas (4, 10); however, many sour orange-rooted trees have remained apparently normal and productive for years following infection by CTV. The failure of CTV to completely decimate the sour-rooted citrus of Florida contrasts markedly with the experience in most citrus areas of the world where CTV has become widespread. The lack of more extensive CTV-induced decline in Florida is not completely understood, but most Florida isolates of CTV are comparatively mild.

[1]This paper reports the results of research only. Mention of a trademark, proprietary product, or pesticide does not constitute a recommendation for its use by the U.S. Department of Agriculture to the exclusion of other suitable products, nor does it imply registration under FIFRA as amended.

Because CTV effects in Florida have been generally mild, use of sour orange rootstock is still widespread. For years, from 30 to 40% of new trees have been propagated on sour orange (5, 10). In the year ending June 30, 1979, more than 525,000 registered trees of 9 varieties (Table 1) were propagated on sour orange. More than 85% of these were sweet orange varieties.

Table 1. Number of registered trees of different varieties propagated on sour orange in Florida, July 1, 1978 to June 30, 1979.

Variety	No. trees	%
Hamlin orange	198,990	37.8
Red grapefruit	62,411	11.9
Valencia orange	133,159	25.3
Navel orange	59,428	11.3
Pineapple orange	32,279	6.1
Parson Brown orange	15,140	2.9
Marsh grapefruit	14,286	2.7
Temple orange	8,383	1.6
Minneola tangelo	2,171	0.4
Total	526,247	100.0

Because CTV is prevalent throughout the major citrus areas and is vectored by several aphids, budwood trees are not registered free of CTV. Extensive propagation of CTV-infected budwood on sour orange rootstocks was suspected but not documented. The recent development of rapid, sensitive, serological procedures to detect CTV has facilitated the rapid and economical survey of large numbers of trees (1, 2, 3).

In this paper, we report the results from indexing 260 registered trees used as budwood sources for propagating nursery trees on sour orange rootstocks and discuss the implications of CTV infection in budwood sources.

Materials and Methods

Tissue was collected in September 1979 from 260 trees in 7 locations in central Florida. These 260 trees supplied buds for about 438,000 propagations of registered trees on sour orange rootstocks in 1979. We collected fruit and/or young, summer flush tissue from 5 sites on the periphery of each tree. The samples were stored on ice in plastic bags until processed.

Enzyme-linked immunosorbent assay (ELISA). Composite 0.5-g samples of diced tissue were prepared. Tissue samples from fruit consisted of pedicel bark and vascular tissue of the fruit "button." Samples from the vegetative summer flush consisted of diced bark tissue peeled from shoots. The 0.5 g of tissue was placed in a 2.5-cm-diameter glass or plastic tube with 5 ml of PBST-2 PVP buffer (phosphate-buffered saline with 0.05% Tween 20(™) and 2% polyvinylpyrrolidone, m.w. 40,000) (1, 2) and homogenized about 15 seconds with a SDT-Tissumizer(™) (Tekmar Co., Cincinnati, OH 45222). The extracts were filtered through cheesecloth or glass wool. The enzyme-linked immunosorbent assay (ELISA) tests were conducted by the double antibody sandwich method (6) as described previously (1, 2). We used polystyrene Micro Elisa(™) plates (Dynatech Labs, Inc., Alexandria, VA 22314) with round bottom wells. Border wells were not used, and healthy, CTV-infected and buffer controls were included in each plate for reference.

The CTV antiserum used was prepared to unfixed, whole virus *(11)*. *Plates were* scored visually and samples diluted 1:3 with distilled H_2O were read for absorbance at 405 nm (OD_{405}) in a spectrophotometer. Samples were considered positive when the OD_{405} value was 2X the healthy control or exceeded the value of the healthy sample by 0.1 OD units when the healthy value was <0.05. Absorbance values reported are actual values corrected for dilution.

Serologically specific electron microscopy (SSEM). Carbon-stabilized, paralodion-coated grids were floated on diluted antiserum to coat the grid with CTV-specific antibodies (8). The sensitized (antibody-coated) grids were washed and then floated on tissue extracts for 1 hr or more. In some cases, the grids were floated on the same extract prepared for ELISA. In other cases, the extracts were freshly prepared in 0.05 *M* Tris buffer pH 8.0. The grids were washed several times, stained in uranyl acetate (9), rinsed with ethanol, and observed with a Phillips 201(TM) electron microscope at instrument magnification from 7,000 to 20,000X.

Graft inoculation of citrus indicators. Trees that indexed negatively by ELISA or SSEM were indexed by graft inoculation to 'Mexican' lime indicator plants using budwood collected at the time samples were collected for ELISA. In addition, 15 randomly selected trees that tested positive for CTV by serology were indexed on 'Mexican' lime, 'Duncan' grapefruit, 'Eureka' lemon (*C. limon* (L.) Burm. f.), and small sweet orange trees grafted on sour orange rootstocks.

Results and Discussion

Eighty-seven percent of the 228 orange and tangelo trees indexed were found to be infected with CTV. These infected trees had supplied buds for 328,438 nursery trees, 89% of the total trees propagated (Table 2). Only 9% of the grapefruit trees indexed were found to be infected, and these infected trees had supplied buds for 8,624 propagations. Because CTV is readily graft-transmitted, nearly all propagations from infected trees will be infected. Infection rates were high in all round orange varieties surveyed (Table 3). The infection rate in grapefruit was similar to that reported earlier (5), but our sample was limited.

High rates of CTV infection were encountered in all 7 locations surveyed (Table 2), but some differences were observed. In some locations, all trees of a particular selection were found to be infected, but, in some, healthy and CTV-infected trees of the same selection were found. In the latter case, natural infection at the tree site is indicated, whereas where all trees are infected, infection may have originated from propagation or natural spread.

Table 3. Citrus tristeza virus infection in registered Florida citrus budwood propagated on sour orange.

Variety	No. of selections	Infection per selection[z] (%)	Propagations	Propagations infected[y] (%)
Minneola	1	100	2,172	100
Navel	7	50-100	42,574	96
Pineapple	3	75-100	31,164	94
Hamlin	3	77-100	171,654	89
Valencia	10	0-100	99,738	88
Temple	1	86	6,719	86
Parson Brown	1	57	14,130	57
Marsh	2	0-100	13,373	55
Redblush	1	0-29	56,522	2
Total	29	—	438,146	77

[z]Percentage of budwood source trees of each selection found infected.
[y]Assumes all propagations from an infected tree are infected.

We believe the ELISA test was accurate for most trees. Fruit tissue and young flush tissue from 78 trees were tested separately with similar results. Over 90% of the samples rated CTV-positive had OD_{405} readings $>4X$ those of the healthy controls. Secondly, 35 of 36 trees which tested positively for CTV by indexing on 'Mexican' lime were found to be positive by ELISA, and 25 of 26 trees that indexed negatively on 'Mexican' lime also tested negatively by ELISA. 'Mexican' lime tests were done on coded samples without knowledge of ELISA results. The two discrepancies which occurred probably resulted from labeling or recording error.

In 78 CTV-infected trees where fruit button and young flush tissue was tested by ELISA, higher readings were obtained from 72 young flush samples. Results shown in Table 2 are from 2 locations where comparisons were made on the same plate. The fruit button samples gave an average OD_{405} reading of 1.5, while the flush sample gave an average of 3.2. Interestingly, the difference between button and flush bark tissue varied somewhat with location (Table 4). We were somewhat surprised by the relatively weak readings for some samples of pedicel bark and button area since these are generally prime sample sources (3) and had given us high readings in preliminary tests earlier in the summer. Months of hot weather and maturity of the fruit may have affected the readings.

The ELISA readings were generally similar for most varieties (Table 4) although exact comparisons are not possible because differences in CTV isolates are not known. In limited tests, SSEM was less reliable than ELISA for detecting CTV in field samples. We detected CTV particles

Table 2. Number of registered budwood source trees infected with citrus tristeza virus (CTV) per location and number of nursery propagations from these sources.

Location	Sweet orange[z]				Grapefruit			
	Trees indexed	% CTV+	Total propagations	% CTV+[y]	Trees indexed	% CTV+	Total propagations	% CTV+
1	26	61.5	25,690	61.3	4	0	3,550	0
2	59	86.4	172,265	91.0	14	0	39,135	0
3	12	100.0	16,544	100.0	4	50	14,882	49
4	34	97.0	25,257	98.7	—	—	—	—
5	—	—	—	—	8	0	7,726	0
6	91	92.3	123,123	90.3	2	50	4,602	29
7	6	50.0	5,372	66.0	—	—	—	—
All	228	87.3	368,251	89.2	32	9.4	69,895	12.3

[z]Includes 'Temple' orange and tangelo.
[y]Assumes all propagations from infected budwood source tree are infected.

Table 4. Optical density (OD) readings from enzyme-linked immunosorbent assays for fruit button[z] and young, summer flush bark samples of different varieties.

Variety	Location	Vegetative flush No. trees	Vegetative flush OD_{405}	Fruit No. trees	Button OD_{405}
Pineapple	1	4	3.4	4	1.0
"	2	7	3.3	7	2.9
Hamlin	1	15	3.3	15	1.1
"	2	3	2.8	3	3.0
Valencia	1	4	2.2	4	1.1
"	2	4	3.5	1	1.0
Navel	1	7	3.0	4	0.9
Parson Brown	1	5	3.1	4	0.8
Minneola	2	1	2.1	1	2.6
Marsh	2	2	4.2	2	2.0

[z]Fruit button sample contained diced fruit pedicel bark and vascular tissues of the button area.

in 22 of 56 samples considered positive by ELISA. However, most of the samples tested by SSEM were ones that yielded relatively weak ELISA readings.

The ELISA or SSEM tests do not indicate the severity of CTV effects in different hosts. Evaluation of severity of selected isolates has been started on selected indicators. These isolates generally produced mild to moderate, vein-clearing, leaf-cupping, stem-pitting, and stunting symptoms on 'Mexican' lime, but several isolates produced quite strong symptoms. None of 15 isolates indexed on 'Eureka' lemon produced seedling yellows symptoms. Results are not available yet from the young sweet orange trees on sour orange rootstock inoculated with these 15 isolates. We assume, however, that most CTV isolates in the budwood sources propagated repeatedly on sour orange are not extremely severe to trees grafted on sour orange. Severe CTV isolates would cause stunting or decline in young trees on sour orange rootstock and make them undesirable.

Further careful evaluation of bud sources for use with sour orange rootstocks is suggested. Firstly, only bud sources known to carry mild isolates of CTV should be used for propagation on sour orange. Secondly, infection of bud sources with mild isolates of CTV may be beneficial by providing future protection against natural infection with severe isolates (7, 13). Evaluation of mild and protecting CTV isolates can be complex because CTV isolates mild in one host may be severe in others (13), and may vary in protective ability (14). Mildness can be measured fairly readily by inoculating appropriate hosts, but full evaluation of protective ability is hampered in Florida by lack of widespread, severe isolates of CTV and absence of the most efficient vector, *Toxoptera citricida*. To evaluate the protective ability of the CTV isolates found in registered Florida budwood sources under more severe conditions, 5 isolates will be tested this coming year in Hawaii where severe isolates and *T. citricida* are present.

The use of rapid detection techniques such as ELISA and SSEM has markedly improved our ability to study CTV and to develop control strategies efficiently. The availability of this technology is timely because CTV still poses a considerable threat to Florida citrus production.

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