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Prevalence of Severe Strains of Citrus Tristeza Virus in Florida Citrus Nurseries

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Abstract. **Sixty-eight percent of the 'Pineapple', 52% of the 'Navel', 46% of the 'Valencia', 38% of the 'Hamlin', and 0% of the 'Ambersweet' orange *[Citrus sinensis* (L.) Osh.] trees in five Florida citrus nurseries were infected with severe strains of citrus tristeza virus (CTV), as demonstrated by reaction with a monoclinal antibody specific for severe strains of the virus. Severe strains of CTV infected 4%, 46%, 76%, 30%, and 48% of the trees at each of the five nurseries, respectively, indicating a considerable difference in severe strain prevalence among the nurseries. Thirty-five percent of the trees in the scion blocks (budwood source) of the nurseries also contained severe strains of CTV.**

Citrus tristeza virus (CTV) causes economically important diseases wherever citrus is grown (Bar-Joseph et al., 1981; Garnsey and Lee, 1988). The virus can cause a variety of effects, including stunting, slow decline, quick decline, stem pitting, or no symptoms, depending on the virus isolate, environmental conditions, citrus cultivar, and rootstock (Garnsey et al., 1987; Miyakawa, 1987). In Florida, only mild isolates, which cause no obvious symptoms, and severe isolates, which cause stunting or decline, are present. Some Florida severe isolates can cause a moderate seedling yellows reaction (Garnsey, 1990). The Florida severe isolates also cause stunting or decline in citrus on sour orange or *Citrus macrophylla* Bunge rootstock; they have not been reported to induce severe stem pitting of scions or cause severe symptoms in citrus on CTV-tolerant rootstock.

CTV-induced disease can be controlled in Florida by propagating citrus on rootstock other than sour orange *(C. aurantium* L.); however, CTV remains a major problem for the Florida citrus industry. First, some sour orange is still being planted, because alternative rootstock are not as tolerant of water stress, citrus blight disease, or phytophthora root rot, or they are not as productive as sour orange on some soil types. Second, there are many productive older groves on sour orange rootstock. These older groves will be in jeopardy if decline-inducing CTV strains are introduced. The real danger exists when replants, propagated with budwood infected with severe CTV and on tolerant rootstock, are placed in groves on sour orange rootstock or in a neighboring grove. These infected replants, which will not show the severe effects of the virus, serve as a reservoir for the severe isolates of CTV.

The virus status of Florida's nursery trees, therefore, is important to growers having a substantial investment in citrus on sour orange rootstock. For this reason, we analyzed trees from five of Florida's citrus nurseries for severe strains of CTV using monoclinal antibodies (Permar et al., 1990; Powell et al., 1992; Vela et al., 1986).

Five large Florida citrus nurseries, capable of producing 500,000 trees annually, and representing the south-central and southeastern regions of the state, were designated A, B, C, D, and E, and sampled three times. The first sampling, in Nov. 1990, consisted of randomly collecting leaf tissue from each of 10 unbudded, 6-month-old seedlings of the rootstock Swingle *(C. paradisi* × *Poncirus trifoliata* Raf. cv. Swingle), Carrizo *(C. sinensis* Osb. × *P. trifoliata* Raf. cv. Carrizo citrange), and Cleopatra *(C. reshni* Hort. ex Tan, cv. Cleopatra mandarin) from each nursery.

The second sampling, in Feb. 1991, consisted of collecting leaf tissue from 10 each of the 9-month-old budded sweet orange *[C. sinensis* (L.) Osb.] cultivars Hamlin, Navel, Pineapple, Valencia, and Ambersweet *[C. reticulata* Blanco × *(C. paradisi* Macf. × *C. reticulata)* × *C. sinensis* (L.) Osb.] from each nursery.

The third sampling consisted of collecting leaf tissue from each of 20 selected trees (one to 12 selections from each cultivar) in the scion blocks (source of budwood) of each nursery in May 1991. The number and cultivars of trees selected were representative of the scion blocks of each nursery.

The nurseries did not obtain all of their budwood from their own scion blocks, and the scion blocks did not contain all the cultivars propagated. All samples (young leaf flush) were placed in ice, transported to the laboratory in Fort Pierce, and stored frozen.

The samples were processed for indirect double-antibody sandwich (DAS)–enzyme-linked immunosorbent assay as described by Powell et al. (1992). Each sample was tested with two monoclinal antibodies: MCA13, which reacts with most Florida severe, but not mild, CTV isolates (Permar et al., 1990), and 3DF1, which reacts with all Florida CTV isolates (Vela et al., 1986).

All of the Swingle, Carrizo, and Cleopatra rootstock seedlings in the five nurseries from the first (November) sampling were free of mild and severe strains of CTV, although potential vectors *(Aphis gossypii* Glover) were present on some of the seedlings, and some of the nurseries were <2 km from known CTV-infected groves.

Thirty-eight percent of the 'Hamlin', 46% of the 'Valencia', 52% of the 'Navel', and 68% of the 'Pineapple' sweet orange from the nurseries reacted with the severe strain-differentiating MCA13 monoclonal antibody and the nondiscriminating 3DF1 monoclinal antibody, showing that severe strains of CTV are very prevalent in budlings in Florida's citrus nurseries (Table 1). These percentages could be an underestimate of the prevalence of severe strain infection, since the MCA13 antibody does not react with every Florida severe strain of CTV (Powell et al., 1992).

None of the 'Ambersweet' oranges in any of the nurseries contained strains of CTV that reacted with MCA13 antibody, indicating that MCA13-reactive severe strains of CTV are not yet prevalent in this cultivar. However, most of the 'Ambersweet' reacted with the 3DF1 antibody, which indicates that other CTV strains were prevalent. 'Ambersweet' is a sweet orange hybrid produced from a 1963 cross between 'Clementine' mandarin and 'Orlando' tangelo (Hearn, 1989). Budwood has not been readily available until recently (1989), which may explain the lack of severe strains in this cultivar.

Most of the nursery trees that did not react with the MCA13 antibody did react with the 3DF1 antibody, indicating that most of the trees that did not contain severe strains of CTV contained milder strains. Most of the trees that contained severe strains probably also contained mild strains, but this supposition cannot be confined since an antibody that reacts with only mild strains of CTV is not available. Trees can be doubly infected with severe and mild strains of CTV (Powell et al., 1992). These results also confirm earlier work with polyclonal antibodies, which indicated that CTV is prevalent in registered Florida budwood sources (Garnsey et al., 1980).

The percentage (4% to 76%) of nursery trees containing severe. strains of CTV varied among the five nurseries. These differences could not be correlated with any particular

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Table 1. Percentage of citrus trees in five Florida nurseries infected with mild or severe strains of citrus tristeza virus (CTV).

Nursery	Cultivar	CTV strain incidence[z] Severe (%)	Mild (%)	Virus negative (%)
A	Ambersweet	0	80	20
	Hamlin	0	80	20
	Navel	0	80	20
	Pineapple	0	100	0
	Valencia	20	50	30
B	Ambersweet	0	90	10
	Hamlin	30	60	10
	Navel	100	0	0
	Pineapple	50	50	0
	Valencia	50	40	10
C	Ambersweet	0	100	0
	Hamlin	100	0	0
	Navel	100	0	0
	Pineapple	90	10	0
	Valencia	90	0	10
D	Ambersweet	0	40	60
	Hamlin	20	30	50
	Navel	0	80	20
	Pineapple	100	0	0
	Valencia	30	50	20
E	Ambersweet	0	80	20
	Hamlin	40	50	10
	Navel	60	40	0
	Pineapple	100	0	0
	Valencia	40	50	10

[z]Percentage of ten 9-month-old trees, randomly collected from Florida citrus nurseries, that were not infected or positive for severe or mild CTV strains, based on enzyme-linked immunosorbent assay reactions with monoclonal antibodies.

Table 2. Number of citrus scion block trees in Florida nurseries infected with mild or severe strains of citrus tristeza virus (CTV).

Nursery	Cultivar tested	No. trees tested	CTV strain incidence[z] Severe	Mild	Virus negative
A	Hamlin	8	1	5	2
	Parson Brown	4	0	3	1
	Valencia	8	5	3	0
B	Ambersweet	2	0	2	0
	Hamlin	5	5	1	4
	Navel	2	0	0	2
	Valencia	11	5	5	1
C	Hamlin	3	3	2	0
	Parson Brown	3	3	0	0
	Pineapple	3	3	0	0
	Valencia	11	11	4	4
D	Hamlin	5	5	3	1
	Navel	3	3	3	0
	Valencia	12	12	4	0
E	Ambersweet	1	1	1	0
	Hamlin	6	6	0	6
	Pineapple	3	3	3	0
	Valencia	10	10	0	5

[z]Number of trees testing negative or positive for severe or mild CTV strains, based on enzyme-linked immunosorbent assay reactions with monoclonal antibodies.

cultural practice or degree of isolation from infected citrus groves. The differences most likely result from the budwood sources, but this could not be confirmed experimentally because the budwood of individual trees could not be traced back to the source, and many nurseries obtain budwood from several locations, in addition to their own scion blocks.

Severe strains of CTV infected 30%, 50%, 45%, 25%, and 25% of the scion block trees at the five nurseries, indicating that the scion blocks are a major source of the severe CTV strains prevalent in Florida citrus nursery stock (Table 2). However, 26% of the scion block trees remained free of CTV. These were usually younger trees from the Florida Dept. of Agriculture budwood program. One-fourth of the scion block trees likely remained free of CTV because of the inefficiency of the predominant CTV vector, *A. gossypii*, in Florida. If the more efficient vector, *Toxoptera citricida* (Kirkaldy), becomes established in Florida, it may become more difficult to keep scion block trees free of CTV.

Beginning in 1963, trees in Florida's budwood certification program were still considered registered even though they were CTV positive. CTV indexing had been ignored because mild strains were endemic in Florida. The surveys clearly showed that strains of CTV that cause severe stunting or decline in sweet orange or grapefruit on sour orange rootstock are now prevalent in Florida's citrus nurseries. This situation presents a real threat to those regions of Florida where sour orange is still the predominant rootstock. Budwood from Florida also may be a source for introducing severe strains of CTV to other regions of the world where sour orange rootstock is widely planted. Additionally, since Florida regulatory and control procedures have failed to prevent the establishment of sour orange-specific stunting and decline strains of CTV in Florida's nurseries, they also may fail to protect nurseries from propagating exotic CTV strains that affect citrus regardless of the rootstock. Several Florida nurseries are working with the Florida Dept. of Agriculture to establish "safe" blocks that are screened to prevent aphid entry and monitored regularly for CTV by grafting to indicators and by monoclinal antibodies.

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