

Prevalence of Citrus Tristeza Virus in Florida Citrus Nurseries and Scion Groves

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Abstract. None of 4190 sweet orange [*Citrus sinensis* (L.) Osb.] nursery trees of ‘Hamlin’, ‘Midsweet’, ‘Navel’, and ‘Valencia’ sampled from five Florida citrus nurseries were infected with a decline-inducing isolate of citrus tristeza virus (CTV) as judged by enzyme-linked immunosorbent assay (ELISA) using isolate-specific monoclonal antibodies. Two of the nurseries had a relatively high level of infection (37% to 100% of composite samples containing tissue from 10 trees) with nondecline-inducing (mild) isolates of CTV, depending on the cultivar. Three of the nurseries had a lower incidence of mild CTV (0% to 22% of 10 tree composite samples). No nursery was CTV-free. ELISA of individual trees used as budwood sources by the nurseries revealed that one tree out of 260 tested contained decline-inducing CTV, and 83 contained mild CTV. These results suggest that the budwood certification program adopted in 1997 has virtually eliminated decline-inducing CTV from commercial budwood supplies.

Citrus tristeza virus (CTV) causes economically important disease worldwide wherever citrus is grown (Bar-Joseph et al., 1981, 1989; Garnsey and Lee, 1988). The virus causes a variety of field symptoms including slow decline, quick decline, stunting, and/or stem-pitting of the scion depending on the virus isolate, host, environmental conditions, and age of the tree when infected. The first three symptom types occur in citrus grafted onto sour orange (*Citrus aurantium* L.) or related rootstocks, while the stem-pitting symptoms mostly occur in sweet orange, grapefruit (*C. paradisi* Macf.), or both, regardless of the rootstock.

Probably the most effective means of reducing CTV-induced disease is through a certification program in which budwood source trees are tested for and maintained free of disease-inducing CTV. Such a program requires regular analysis of the source trees using reliable, rapid, and low cost techniques. The technique that best meets these criteria, and is used in most certifications, is enzyme-linked immunosorbent assay (ELISA). In Florida, the assay is performed using the decline isolate-specific monoclonal antibody (MAb) MCA13 (Permar et al., 1990). This MAb reacts with most decline-inducing CTV in Florida, but does not react with nondecline-inducing CTV (Permar and Garnsey, 1991). The MCA13 based discrimination between decline- and nondecline-inducing isolates of CTV outside of Florida may not be effective (Cambra et al., 1993).

In 1993, it was reported that between 4% and 76% of the trees in five Florida citrus nurseries, and 35% of Florida budwood source trees tested, were infected with decline-inducing CTV (Powell and Pelosi, 1993). As a result

of reports like this and concerns about the introduction of stem-pitting CTV into commercial citrus, a mandatory budwood program was established in Florida in 1997. This program required that commercial citrus nursery stock in Florida be propagated from certified trees which had assayed negative for decline-inducing CTV by ELISA using MCA13.

It was hoped the program would reduce the incidence of severe CTV in Florida’s nursery stock and budwood sources. We have conducted another survey to test this hypothesis.

Materials and Methods

Five Florida citrus nurseries, capable of producing 500,000 trees annually, and representing the south-central and southeastern regions of the state, were selected and designated A, B, C, D, and E. Three of these nurseries (A, B, and D) were the same as those sampled in 1991 (Powell and Pelosi, 1993). At each nursery, samples were taken from both their nursery stock (9-month-old budded sweet orange trees ready for sale) and their scion block (trees that were used directly as budwood sources or were used as the source of buds for increase blocks which were in turn used as budwood sources for nursery stock). The sweet orange cultivars sampled were ‘Hamlin’, ‘Midsweet’, ‘Navel’, and ‘Valencia’. In three nurseries, the ready-for-sale stock was grown in greenhouses; at the other two nurseries, the stock was planted in the field. One nursery kept its scion block in an insect-free screenhouse, three nurseries had their scion block in the field, and one nursery had scion trees in both a screenhouse and in the field.

Sampling of the nursery stock followed the procedures for sampling a percentage of the trees and assaying bulked samples as outlined by Hughes and Gottwald (2001). Greenhouse container-grown nursery stock was sampled by walking around the perimeter of the greenhouse

bench, collecting a twig of the youngest tissue from every 4th to 6th tree depending on the number of trees in each bench row. Ten twigs from 10 consecutive samplings were combined and used as the assay tissue. Field-grown nursery stock was sampled by selecting two rows, at random, from each block and walking down the row, collecting a young twig from every 10th tree. Ten twigs collected from consecutive collections were combined and used as the assay sample. Control assay samples were one twig from a T36 (severe CTV), T30 (mild CTV), or uninfected sweet orange tree combined with nine twigs from uninfected trees. Container-grown and field-grown nursery stock were replicated by bench or row, respectively.

Scion blocks were sampled by collecting five twigs from different regions of the canopy of each tree. These five twigs were combined and used as the assay sample. Twigs from different trees were not combined. Control assay samples were five similarly collected twigs from T36, T30, or uninfected trees.

About 0.1 g of each twig, in each composite sample, was processed using a Kleco pulverizer (Kinetic Laboratory Equipment Co., Visalia, Calif.) in 7.5 mL of TPBS buffer (0.015 M sodium phosphate 0.15 M NaCl, 0.05% Tween 20, pH 7.4). ELISA was performed as previously described (Powell et al., 1992), except monoclonal antibody 17G11 was used as the strain nonspecific second antibody.

Results

Nursery stock. Many of the nursery trees (30% of 10-tree composite samples had at least one tree infected) were infected with a nondecline-inducing (mild) isolate CTV as shown by reaction with MAb 17G11, but not MAb MCA13 (Table 1). The amount of infection varied considerably among citrus cultivars and among nurseries. For example, the overall percentage of mild CTV infected samples (grouping all cultivars together) from nursery C was 3%, and only in ‘Valencia’, while 88% of the samples collected from nursery D were positive. The results were expected since most nurseries are either indifferent towards the presence of mild CTV isolates or deliberately propagate the mild isolates as potential cross-protecting agents.

None of the 419 10-tree composite samples reacted with MAb MCA13 in ELISA (Table 1). This suggests that of 4,190 nursery trees assayed, all were free of severe CTV isolates detectable by ELISA. This compares to a 4% to 76% (depending on the nursery) severe CTV infection rate reported in 1993 (Powell and Pelosi, 1993).

Scion blocks. ELISA of individual scion block trees used by the five nurseries showed a combined mild CTV infection level of 32% (Table 2). This was similar to the infection rate found in the nursery stock. As with the nursery stock, there was also considerable difference among nurseries. Only one nursery had all its scion trees free of CTV. This nursery kept its budwood in a screenhouse.

Decline-inducing CTV was detected by ELISA using MAb MCA13 in only one bud-

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Table 1. Percentage of samples from five Florida nurseries infected with mild or severe isolates of citrus tristeza virus (CTV).

Nursery	Planting type	Cultivar	No. of 10-tree composite samples assayed	CTV incidence ²		
				Severe ¹ (%)	Mild ¹ (%)	Virus negative (%)
A	Greenhouse	Hamlin	27	0 ± 0	56 ± 4	44 ± 4
		Midsweet	6	0 ± 0	50 ± 0	50 ± 0
		Navel	23	0 ± 0	78 ± 14	22 ± 14
		Valencia	16	0 ± 0	37 ± 15	63 ± 15
B	Greenhouse	Hamlin	20	0 ± 0	0 ± 0	100 ± 0
		Midsweet	20	0 ± 0	0 ± 0	100 ± 0
		Valencia	20	0 ± 0	5 ± 10	95 ± 10
C	Greenhouse	Hamlin	47	0 ± 0	0 ± 0	100 ± 0
		Midsweet	3	0 ± 0	0 ± 0	100 ± 0
		Navel	8	0 ± 0	0 ± 0	100 ± 0
		Valencia	64	0 ± 0	12 ± 5	88 ± 5
D	Field	Hamlin	21	0 ± 0	96 ± 8	4 ± 8
		Midsweet	25	0 ± 0	88 ± 18	12 ± 18
		Navel	7	0 ± 0	100 ± 0	0 ± 0
		Valencia	21	0 ± 0	67 ± 9	33 ± 9
E	Field	Hamlin	33	0 ± 0	21 ± 5	79 ± 5
		Midsweet	21	0 ± 0	0 ± 0	100 ± 0
		Navel	3	0 ± 0	0 ± 0	100 ± 0
		Valencia	34	0 ± 0	18 ± 10	82 ± 10

²Percentage of samples positive for CTV by ELISA. Each sample consisted of a composite collected from 10 different nursery trees.

¹Sample extract reacted with MAb MCA13 by ELISA.

³Sample extract reacted with MAb 17G11 by ELISA, but not by MAb MCA13.

wood source tree out of 247 tested (Table 2). This tree has since been removed.

Discussion

In 1993, it was reported that several of Florida's citrus nurseries had a high level of decline-inducing CTV in their nursery stock and scion groves (Powell and Pelosi, 1993). Since then, a mandatory budwood certification program has been implemented requiring that budwood source trees test negative for decline-inducing CTV by ELISA using MAb MCA13.

There are limitations in detecting decline-inducing CTV by ELISA using MCA13. It has been demonstrated that sweet orange and grapefruit that are infected with both

decline-inducing and nondeclining isolates of CTV may carry the decline-inducing isolate at a concentration or distribution that eludes detection by ELISA using MCA13. The decline-inducing isolate can be detected by other methods such as in situ immunoassay (Lin et al., 2000, 2002) or grafting to indicators (Powell et al., 2003). It is possible that some of the nursery stock and scion grove trees assayed may contain decline-inducing CTV that is not detectable using MCA13 ELISA. This uncertainty must be resolved by further experimentation.

In spite of these drawbacks, our results indicate that this program has been highly successful in reducing the incidence of decline-inducing CTV in Florida nursery stock. No decline-inducing CTV was detected in

Table 2. Infection of scion block trees from five Florida citrus nurseries with severe or mild isolates of citrus tristeza virus (CTV).

Nursery	Planting type	Cultivar tested	No. trees tested	CTV incidence ²		
				Severe	Mild	Virus negative
A	Screenhouse	Hamlin	2	0	0	2
		Midsweet	6	0	0	6
		Navel	2	0	0	2
		Valencia	6	0	0	6
B	Field	Hamlin	25	0	2	23
		Midsweet	25	0	0	25
		Navel	10	0	0	10
		Valencia	25	0	2	23
C	Field	Hamlin	5	0	1	4
		Midsweet	3	0	0	3
		Navel	1	0	1	0
		Valencia	3	0	3	0
D	Field	Hamlin	10	0	10	0
		Midsweet	10	0	9	1
	Screenhouse	Navel	7	1	7	0
		Valencia	29	0	20	9
E	Field	Hamlin	33	0	0	33
		Midsweet	21	0	0	21
		Navel	3	0	0	3
		Valencia	34	0	28	6

²Number of trees testing negative or positive for severe or mild CTV isolates, based on ELISA reactions with monoclonal antibodies.

nursery stock and only one MCA13 positive scion block tree was found. This is in spite of the introduction of the brown citrus aphid (*Toxoptera citricida* Kirkaldy) into Florida.

The fact the one scion grove Navel tree was found to be infected with decline-inducing CTV in nursery C, but no decline-inducing CTV was detected in Navel nursery stock in the same nursery is not contradictory. The infected tree, which was on sour orange rootstock did not appear completely healthy, and the nurseryman informed us it had not been used as a budwood source that year.

Hughes and Gottwald (2001) have developed survey methods for assessment of CTV in citrus nurseries. These methods, which were used in this survey, allowed us to rapidly assess large numbers of trees to derive an overall picture of CTV prevalence in both greenhouse, container-grown, and field-grown nursery stock.

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