Natural Field Spread of Decline and Nondecline Inducing Isolates of Citrus Tristeza Virus in Florida after the Introduction of the Brown Citrus Aphid

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Abstract. The effectiveness of seven different aphid control regimes in delaying movement of decline (DI) and nondecline (NDI) inducing isolates of citrus tristeza virus (CTV) into a CTV-free sweet orange scion on sour orange rootstock block was monitored annually for 5 years beginning in 1999, 2 years after the introduction of the brown citrus aphid (BrCA) into the region. After 5 years, the mean percentages of infection with DI CTV were 19, 19, 17, 29, 23, 19, or 14 for trees treated annually with imidocloprid, every 6 months with imidocloprid, every 3 months with imidocloprid, every 2 months with imidocloprid, annually with Temik, annually with Meta Systox-R, or untreated, respectively. The mean percentages of infection (after 5 years) with only NDI isolates of CTV for the seven treatments were 40, 31, 33, 38, 38, 38, or 33. There was no significant difference (after 5 years) among either the DI or NDI CTV treatment means. The overall 5-year infection percentage for DI CTV (20%) was somewhat lower than that reported before the introduction of the BrCA (27%) (11). Aphid densities (*Toxoptera citricidus* and *Aphis spiraecola*) varied considerably from year to year. Good aphid control was achieved with all four imidocloprid treatments, but not with Temik or Meta Systox-R. The level of aphid control did not influence overall CTV infection percentages.

Citrus Tristeza virus (CTV) causes economically important diseases of citrus wherever it is grown (Bar-Joseph et al., 1981; Garnsey and Lee, 1998). The virus can cause a variety of symptoms; including stunting, slow decline, quick decline, stem-pitting, or undersized fruit, or infected trees may be symptomless (Garnsey et al., 1987). The symptomology is influenced by many factors; including virus isolate, citrus scion cultivar, rootstock, time of infection, and environmental conditions, especially rainfall. In Florida, CTV isolates that do not cause symptoms and those that cause stunting, slow decline or quick decline of citrus on sour orange (*Citrus aurantium* L.) or related rootstocks are common.

The primary vectors of CTV are the brown citrus aphid (BrCA), *Toxoptera citricidus* Kirkaldy, the melon aphid, *Aphis gossypii* Glover, and the green citrus aphid, *Aphis spiraecola* Patch (Abate, 1988; Cambra et al., 1981; Essig, 1949). The latter two were responsible for most CTV transmission in Florida until 1997, when the BrCA was introduced. The rate of transmission and field spread of CTV has been examined in California (Roistacher et al., 1984; Roistacher et al., 1980), Spain (Hermoso de Mendoza et al., 1988), and Florida (Yokomi and Garnsey, 1987; Yokomi et al., 1987). These studies have indicated that there are both highly and poorly transmissible CTV isolates.

In 1997, we reported on the field spread of decline inducing (DI) and nondecline inducing (NDI) isolates of CTV in a field plot at the Indian River Research and Education Center (IRREC) in Fort Pierce, Fla., and the effect of five aphid control regimes on the movement of these viruses (Powell et al., 1997). The overall percentage of trees infected with DI CTV after 5 years was 27. This amount of infection was not affected by any of the aphid control regimes tested (Powell et al., 1997). Since those studies, two phenomena have occurred that can influence CTV transmission in Florida. First, the BrCA was introduced and flourished in the Indian River Region of Florida in 1997, and second, an insecticide, imidocloprid, as shown to reduce whiteflies (Powell and Stoffella, 1998) and aphids (C. Powell, unpublished data) to very low levels in tomatoes and squash.

In 1996, we established another citrus field planting at the same location as the previous study reported in 1997. We have measured the infection of these trees with DI and NDI CTV to determine the influence of BrCA and aphid control regimes, including imidocloprid, on virus movement and aphid populations.

Materials and Methods

CTV-free 'Valencia' sweet orange (*Citrus sinensis* (L.) Osbeck) trees (294) grafted on to sour orange rootstock (*C. aurantium* L.) were planted in June 1997 within 30 m of a 'Temple' orange block that was virtually 100%

infected with NDI and DI isolates of CTV (25 of 25 trees tested were infected with both DI and NDI CTV). The location was the same site as a previously reported study on CTV movement before BrCA introduction into the area (Powell et al., 1997). The test trees were in single beds (rows) with 9.15 m between rows. The between-tree spacing was 4.6 m.

The experiment was a randomized complete block design with each of six rows serving as a replication. Each row had two trees on each end that were not part of the block. There were seven treatments in each of the six replications, with seven trees per experimental unit (plot). The treatments were an annual application of Temik (Rhone-Poulenc, Research Triangle Park, N.C.) (8.5 g a.i./tree, incorporated into the soil); an annual application of Meta-Systox-R (Möbay Corp., Kansas City, Mo.) (trunk drenched, 0.62 mL·L⁻¹); soil drenches with imidacloprid (1-[6-chloro-3-pyridinyl] methyl]-N-nitro-2-imidazolidinimine) (Bayer, Vero Beach, Fla.) at 1920 mg a.i./plant applied once a year or every 6, 3, or 2 months; and no insecticide application. Temik was applied the last week of April, and the trunk drenches were applied in the spring between 19 Apr. and 22 May. Young flush was sampled at three locations on each tree during the winters of 1999 (January to February) through 2003. The three samples were analyzed individually for DI and NDI isolates of CTV by direct tissue blot immunoassay (DTBIA) as previously described (Lin et al., 2002), using monoclonal antibodies 17G11 and MCA 13 (Permar et al., 1990). Numbers and species of aphids were monitored monthly by counting the total number in the leaf canopies of each of the seven trees in each plot.

Infection percentages and aphid numbers (square root transformed) were subjected to an analysis of variance (ANOVA) by the SAS software program (SAS Institute, Cary, N.C.). Main treatment effect means that had a significant F test were separated by Duncan's multiple range test, 5% level.

Results

The percentage of untreated trees (no aphid control) infected with DI CTV (reacted with MAb MCA 13) after one, two, three, four, or five growing seasons between 1999 and 2003, after the BrCA was introduced into the area, was 9, 12, 12, 12, or 14, respectively (Table 1). Previously reported (Powell et al., 1997) 5-year (1990–94) DI CTV infection percentages (before BrCA introduction) were 0, 0, 7, 7, or 28 (Table 1). In the post-BrCA experiments most of the DI CTV infection occurred early during the first 2 years. In the pre-BrCA experiment most of the DI CTV infection occurred late during the last 2 years. Neither the post-BrCA nor the pre-BrCA control strategies were effective in reducing DI CTV transmission ($P \leq 0.05$). There was no significant difference among blocks ($P \leq 0.05$).

The percentage of untreated trees (no insecticide) infected with only NDI CTV (reacted with MAb 17G11, but not MAb MCA13) after one, two, three, four, or five growing

Table 1. The spread of decline-inducing (DI) and nondecline-inducing (NDI) isolates of *Citrus tristeza virus* (CTV) before and after the introduction of the brown citrus aphid (BrCA)^z.

Aphid control ¹	CTV infection (%) ^y									
	NDI ^v		DI		NDI		DI		NDI	
	DI ^w		DI		DI		DI		DI	
	1990		1991		1992		1993		1994	
Temik	0	0	0	0	10	3 a	10	5 a	32	13 ab
Temik + MSR ^u	0	0	0	0	3	3 a	5	3 a	32	10 ab
MSR	0	0	0	0	3	8 ab	3	8 ab	20	7 a
Stylet oil	0	0	0	2	8	2 a	8	7 ab	25	7 a
Untreated	0	0	0	2	7	12 b	7	13 b	28	17 b
	1999		2000		2001		2002		2003	
Imidacloprid 1X	5	19	12 a	14 ab	12 ab	29 ab	12	38	19	40
Imidacloprid 2X	7	4	17 ab	7 a	19 ab	14 ab	19	21	19	31
Imidacloprid 4X	5	10	5 a	17 ab	17 ab	14 ab	17	26	17	33
Imidacloprid 6X	12	12	24 b	24 b	24 b	31 b	24	36	29	38
Temik	7	17	12 a	21 b	12 ab	21 ab	14	21	23	38
MSR	0	7	7 a	10 a	7 a	9 a	9	26	19	38
None	9	5	12 a	7 a	12 ab	12 ab	12	23	14	33

^zData from 1990–94 have been previously published (Powell et al., 1997).

^yMean of six replications with seven trees per replication. Based on direct tissue blot immunoassay with monoclonal antibodies that can differentiate DI and NDI isolates. Mean separation by Duncan's multiple range test, 5% level.

^x1X, 2X, 4X, 6X = applied once a year, every 6 months, every 3 months, or every 2 months, respectively.

^wSample reacted with MAb MCA13.

^vSample reacted with MAb 17G11, but not MCA13.

^uMeta Systox-R.

Table 2. Mean aphid numbers in a sweet on sour orange tree block treated with various insecticides.^z

Treatment ^y	1998		1999		2000		2001		2002	
	BrCA ^x	AS ^w	BrCA	AS	BrCA	AS	BrCA	AS	BrCA	AS
Imidacloprid 1X	4 a	516 a	3 a	559 a	3	10	34 a	8	0 a	1 a
Imidacloprid 2X	0 a	4 a	0 a	0 b	1	6	2 a	2	2 a	0 a
Imidacloprid 4X	1 a	14 a	0 a	9 b	0	0	0 a	1	0 a	2 a
Imidacloprid 6X	0 a	5 a	0 a	0 b	0	0	2 a	0	0 a	0 a
Temik	273 b	18352 b	92 b	1162 a	57	71	1301 bc	2	123 b	22 a
MSR	2486 c	3695 c	1790 c	1413 a	12	0	2235 c	17	112 b	3 a
None	373 b	5227 c	118 b	1144 a	75	3	767 bc	2	75 b	172 b

^zNumbers are the average of the total aphids counted in each seven-tree plot during each year (counts were made once a month). There were 6 replications of each treatment. Mean separation by Duncan's multiple range test, 5% level.

^x1X, 2X, 4X, 6X = applied once a year, every 6 months, every 3 months, or every 2 months, respectively.

MSR = Meta Systox-R.

^w*Toxoptera citricidus*.

^y*Aphis spiraecola*.

seasons (1990–2003), was 5, 7, 12, 23, or 33, respectively (Table 1). This compares to previously reported (Powell et al., 1997) 5-year NDI CTV percentages of 0, 2, 12, 13, or 17 (Table 1). Again, aphid control strategies did not reduce NDI CTV infection ($P \leq 0.05$). There was no significant difference among blocks ($P \leq 0.05$).

Aphid populations varied considerably from year to year, with 95% of all aphids occurring between September and December. Moderate populations of both BrCA and *Aphis spiraecola* (AS) were counted during 1998 and 1999 and BrCA reappeared in 2001 (Table 2). Aphids were difficult to find in 2000 and 2002. *Aphis gossypii*, which was found between 1989 and 1994 (Powell et al., 1997), was rare between 1998 and 2002. Large aphid infestations 1 year appeared to result in an increased number of detectable CTV infections the following year. For example, large numbers of aphids were counted in 1998 and there were many CTV infections detected in 1999. There were few aphids counted in 2000 and relatively fewer new CTV infections detected in 2001. The largest aphid numbers occurred on Temik-treated trees in 1998. Temik may increase young tree growth providing more young tissue suitable for aphid feeding (R.

Pelosi and R. Bullock, unpublished observations). All the imidocloprid treatments gave adequate aphid control, but subsequently did not reduce CTV infection.

Discussion

The introduction of the BrCA did not lead to an increased rate of infection by CTV in the Indian River Region of Florida. This is in contrast to observations made in other parts of the world (Roistacher and Bar-Joseph, 1987) or even in some parts of Florida (Powell, unpublished observations). The reason that the predicted post-BrCA increase in CTV infection in the Indian River Region of Florida did not occur may be due to the reported low transmission efficiency of local CTV isolates by local BrCA cultures (Lin et al., 2000), and in the failure of the BrCA to maintain large population densities (Table 2). The year after the BrCA was introduced it was difficult to find young citrus leaves in the field without aphids, however, since that time populations have been sporadic. The reasons for the low sporadic populations of BrCA, and the virtual disappearance of *Aphis gossypii* are unknown but may be related to natural and released predators and parasites. At any rate, the introduction

of BrCA does not necessarily translate into increased CTV infection.

The insecticide treatments did not decrease CTV infection, even under the adequate aphid control attained with the imidocloprid treatments. Imidocloprid has been successfully used for several years to control whitefly vectored geminiviruses of tomatoes in Florida (C. Powell, unpublished observations) even though the whitefly and geminivirus field transmission is far more efficient than CTV/aphid transmission (C. Powell, unpublished observations). The reason for this discrepancy is unknown. However, in the geminivirus control strategy the imidocloprid is being applied to large acreages of adjacent fields while in our experiment the imidocloprid was only applied to a small block and adjacent citrus was untreated. Perhaps regional applications of imidocloprid could control CTV. This would likely not be practical at current fruit prices.

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