Effect of Suppression by Tree Removal on the Incidence of Citrus Tristeza Virus in California

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ABSTRACT. Incidence of citrus tristeza virus (CTV) in 21 groves of sweet orange on quick decline-tolerant rootstocks was monitored for a 3-yr-period to assess CTV suppression by rapid removal of infected trees. Determinations of CTV infection were made by testing tissue extracts from all trees in each grove by ELISA. The average number of infected trees detected in a grove in a single testing period was 68 (5.5% of all trees). Approximately one year following tree removal, the average number of infected trees dropped to 42 (3.7%). The following year, infected trees were not removed and the average number of infected trees increased to 64. In five of these sites there was an extra year of CTV eradication and the average number of infected trees was reduced from 262 to 98 after the first year and then to 62 the second year (a 77% suppression). In the third year, infected trees were not removed and the average number of infected trees increased to 93. These results indicated that more than two years of repeated tree removal are required to approach eradication and that the benefit of an entire year of suppression can be lost due to natural spread by aphid vectors when inoculum removal is interrupted.

Index words. ELISA, eradication, *Aphis gossypii.*

Citrus tristeza virus (CTV) is managed in different parts of the world by the use of quick decline-tolerant rootstocks (e.g. citrange), use of clean virus-tested stock, and/or the use of cross protection (1). In areas where overall CTV incidence is low, management by suppression of the virus, with the long term aim of eradication, may be a viable option if the rate of infected tree removal exceeds the rate of new infections.

Management of CTV by eradication is practiced in the counties of Fresno, Tulare, and Kern located in the Central Valley of California (4). A recently completed survey of all groves in the eradication area suggests that 89% of the groves are negative for CTV (3, CCTEA, unpublished data). Only those trees that test positive for CTV by ELISA in two independent assays are removed. Once tree removal has occurred, every tree in that grove is retested annually and any additional infected trees are removed. *Aphis gossypii* Glover, the cotton or melon aphid, is the major natural vector of CTV in California (2) and is a common aphid species in the eradication area. The results presented here indicate that the virus is spreading throughout the Central Valley by aphid transmission.

This report describes and analyzes the effect of periodic (approximately annual) tree removal on the overall incidence of CTV in 21 selected groves. The data for one period (21 groves) or two periods (5 of these groves) of suppression are presented. The last date for which new ELISA data are presented is Spring, 1995. During the fiscal year 1994-95, a moratorium on tree removal was implemented. This break in the suppression effort was used to estimate the effect of one year of no tree removal on the management of CTV by suppression in the selected groves.

MATERIALS AND METHODS

All sampling and testing of sweet orange trees on quick decline tolerant rootstocks was done at the Central California Tristeza Eradication Agency (CCTEA). The groves selected for this study were sampled in the 1990/91, 1991/92, or 1992/93 periods. Samples were collected from all trees. A sample consists of a bun-

TABLE 1
EFFECT OF SUPPRESSION AND A MORATORIUM YEAR ON THE INCIDENCE OF CTV IN SWEET ORANGE GROVES IN THE CENTRAL VALLEY, CALIFORNIA

Grove	Initial tree number	Initial CTV incidence (%)	CTV incidence Period 3 (%)	No of CTV-positive trees at each period with or without suppression[z]			
				Yes 1	Yes 2	No 3	Pending[y] 4
1	2,600	26.0	7.9	676	351	206	240
2	2,094	15.5	2.4	324	45	50	100
3	3,004	7.2	1.2	215	53	37	103
4	836	6.0	0.7	50	20	6	7
5	836	5.3	1.6	44	19	13	15
6	595	8.4	2.2		50	13	26
7	152	7.9	4.6		12	7	15
8	1,170	7.7	3.5		90	41	79
9	672	7.6	4.5		9	4	8
11	515	6.6	8.7		34	45	57
12	1,181	6.4	1.5		76	18	35
13	967	6.4	4.9		62	47	70
14	3,157	6.2	3.7		197	117	150
15	838	6.1	1.6		51	13	28
16	275	5.8	12.4		16	34	48
17	1,018	5.1	3.4		52	35	62
18	1,071	5.0	1.5		54	16	32
19	1,467	4.2	3.5		61	51	79
20	1,588	4.2	1.7		66	27	63
21	1,936	3.4	3.7		66	71	79
Avg (1-5)	1,874	12.0	2.8	262	98	62	93
Avg (1-21)	1,243	7.5	3.7		68	42	64

[z]Yes = CTV-positive trees removed; No = CTV-positive trees not removed due to a grower- supported moratorium on tree removal; Pending = CTV-positive trees marked for removal post-moratorium

[y]Period 1 = 1990/91; Period 2 = 1991/92; Period 3 = 1992/93; Period 4 = 1993/94

dle of 8 leaves, collected from all four sides of a tree. The petiole and the base of the leaf are cut from the bundle and processed with a grinder. Sap is tested by ELISA (3, 5).

Samples with and OD_{405} value of 2.5 times that of the non-infected controls (or higher) were considered positive for CTV. Any tree that tested positive was resampled and trees positive in both tests were targeted for tree removal. During the following year, the grove was revisited and all trees were re-tested as described. All newly discovered infected trees were removed. This process is usually repeated until CTV has been suppressed in the grove. An exact twelve months between sampling dates was not always possible in the groves used for this study. The intervals between sampling dates were therefore called periods in this report and varied between 11 and 18 mo. Periods 1 through 4 correspond to the seasons 1990/91 through 1994/95. The sampling times for Periods 3 and 4 were Spring, 1994 and Spring, 1995, respectively.

The criteria for selection of groves were that at least one round of suppression had taken place and that each sampling date for ELISA was during a month when ELISA titer was high in the tree (5). Twenty one groves met these criteria. Five of these groves had two and the remainder had one period of suppression.

RESULTS

The number of trees in the groves varied from 133 to 3,157 with an average of 1,243 trees. The initial incidence of CTV in the groves ranged from 3.4 to 26.0%, with an average of 7.5%. The five groves where suppression was practiced twice (Table 1, groves 1 through 5) had an average of 1,874 trees and an average initial disease incidence of 12%. Table 1 is sorted by initial disease incidence, groves 1 through 5 first (groves with two periods of suppression) and then groves 6 through 21 (groves with one period of suppression).

In groves 1 through 5, which all had two cycles of suppression (Periods 1 and 2), the average number of infected trees was reduced from 262 to 98 in the first cycle and then to 62 at the end of the second cycle (a 77% suppression over 2 periods). One period later, with no further tree removal (the moratorium period), the average number of infected trees increased to 93 (Fig. 1).

At 21 sites the average number of infected trees was 68 (5.5% of all trees) at the beginning of Period 2. This is a time either prior to any tree removal (groves with one period of suppression) or after one period of suppression (the five groves that had two periods of suppression). An average of 42 infected trees (3.7%) were present one period later (Period 3). These trees were not removed due to the moratorium period and, as a result, the average number of infected trees increased to 64 after the next period (Period 4).

There are 26 examples of periodic suppression in Table 1. Detection of fewer infected trees at the end of the period than were originally removed at the beginning of the period occurred in all but 3 examples (groves 2, 11, and 16).

Disease/pathogen progression of CTV in the absence of suppression can be estimated from the data for Periods 3 and 4 (the moratorium period) for this set of 21 groves. The average incidence increased from 42 to 64 infected trees, a 52% increase during the period Spring, 1994 to Spring, 1995. An increase was detected in all of the 21 groves.

DISCUSSION

In Central California, it was possible to suppress CTV in some groves of sweet orange trees that had an initial incidence of about 10% infection. This was accomplished by periodic (approximately annual) testing of groves for CTV by ELISA followed by quick removal of infected trees. The results indicated that more than two years of suppression are required to approach eradication and that the benefits of an entire year of suppression can be lost to

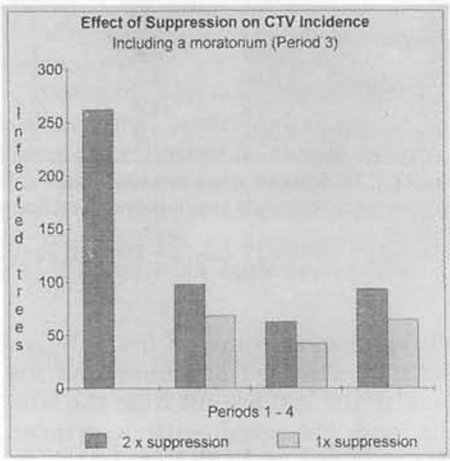

Fig. 1. Effect of suppression (removal of all serologically detected CTV positive trees) on CTV incidence in California groves of sweet orange trees on quick decline tolerant rootstocks. The data presented include those from a moratorium period when no eradication occurred. Data is for 21 groves which had one cycle of suppression in period 2 (light shading). Five of them had an additional earlier cycle of suppression in period one (dark shading). Changes in the number of CTV positive trees is compared for each period. Suppression occurred during period 1 and 2, but no suppression was practiced during period 3. The duration of each period is between 12 and 18 mo.

transmission when annual tree removal is interrupted.

Because infected trees were removed, it is not known what the relative disease/pathogen progression rates for the virus were in these groves for each of the periods described. The only year for which this information is available is after the moratorium year (Period 4), when CTV infection level increased by 52% from the previous year. This is not an unexpected rate of progression for CTV when *Aphis gossypii* is the vector (6). It is assumed that the rate of disease progression varies from year to year due to fluctuations in vector aphid populations and behavior. The ability of the CCTEA to practice consistent periodic suppression is in contrast to erratic disease progression and suggests that it is possible to match or exceed the ability of *Aphis gossypii* to transmit CTV to uninfected remaining trees in Central California by repeated annual tree removal. It is still not clear what the periodicity should be in order to obtain maximum benefits from suppression, nor whether periodicity of suppression should vary from grove to grove to obtain the same benefit. It is also unclear what criteria should be used to determine when to stop testing and removing trees from any given grove.

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