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Journal

International Organization of Citrus Virologists Conference Proceedings (1957-2010), 12(12)

ISSN

2313-5123

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Publication Date

1993

DOI

10.5070/C504b0n63q

Peer reviewed

Analysis of Epidemics of Citrus Tristeza Virus (CTV) in Young Citrus Groves Exposed to Aphid Infestation under Different Climatic Conditions in Reunion Island

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ABSTRACT. Citrus Tristeza Virus (CTV) is widespread in Reunion Island where *Toxoptera citricidus*, the most efficient vector and various other citrus aphids are present. Severe strains of the virus that affect the longevity and productivity of susceptible citrus species have been reported. In order to monitor the natural spread of CTV under different ecological conditions, various experimental citrus blocks were planted with tristeza-free nursery plants in four locations, and citrus aphids were recorded on young flushes.

The percentage of infected trees one year after planting varied from 21% to 100%, in six orchards in which ELISA was used for detection of CTV. Spatial distribution of diseased trees within citrus blocks was generally random. The predominant aphid infesting young lime trees at low elevation (150 m) was *T. citricidus*, while *Aphis* spp. and *Macrosiphum euphorbiae* Thomas were more frequent at higher elevation (800 m).

This paper presents information on the effect of different environmental factors on spread of CTV.

Citrus tristeza virus (CTV) and its most efficient vector *Toxoptera citricidus* Kirkaldy were first reported in Reunion Island at the end of the 1960's (3,9). At that time tristeza was well established, suggesting previous introduction of both the virus and the vector to Reunion, where they are presently considered endemic. The citrus industry of the island is estimated at 400 ha, divided into numerous small private orchards (50 to 200 trees), located in diversified ecological environments ranging from sea level to 1000 melevation. Sweet orange and mandarin are the main cultivated citrus species. However, various species that are more suceptible to CTV, such as Mexican lime, combava, or grapefruit are increasing in cultivation due to the high demand of these fruits in the local market.

The presence of severe isolates of CTV limits the choice of rootstocks and strongly affects productivity of CTV-suceptible species (1,7). All major potential vectors of CTV reviewed by Raccah *et al.* (14), were identified in Reunion (15). However, population dynamics of these species in citrus orchards has not been studied.

The purpose of this research was to study the spread of CTV within newly established citrus groves in different locations in Reunion.

MATERIALS AND METHODS

Planting material. Young trees of the following varieties were produced in the screenhouse from CTV-free budwood introduced from the Station de Recherche Agrumicole of San Giuliano : Mexican lime "Sans épines", combava, Valencia Late Rhodes red orange SRA 360, Washington Navel orange SRA 217 and various clementine cultivars such as Commune SRA 63, Commune SRA 92, Monreal GA 137, 2000 SRA 272, Oroval SRA 340, Nules SRA 334, Corsica 2. All these trees were grafted on Carrizo citrange.

Six citrus orchards were established at different locations as indicated in Table 1. Orchards were planted on a 6 m x 4 m pattern and managed as recommended by the extension service of CIRAD-IRFA.

Aphid infestations were recorded in the orchards of Bassin Plat and Colimaçons. Trees were sprayed with aphicides when more than 25% of the trees hosted at least one aphid colony.

CTV detection. Trees were assayed individually by enzyme-linked immunosorbent assay (ELISA) every 4 to 6 months. Bark extracts from three twigs/plant were assayed using a polyclonal antibody (Sanofi Santé Animale, Libourne, France) in double antibody

TABLE 1
DESCRIPTION OF THE EXPERIMENTAL CTV-FREE ORCHARDS, PLANTING DATE, NUMBER OF TREES AND CLIMATOLOGICAL DATA.

Species	Location	Altitude (m)	Planting date	No. of trees	Daily average temperature (C)		Annual rainfall (mm)
					August	Febraury	
Mexican lime	Bassin Plant	150	Oct 90	90	19.6	25.6	1100
Clementine	Colimaçons	800	Jan 91	63	15.8	21.6	1450
Valencia	Colimaçons	800	Jan 91	54	15.8	21.6	1450
Combava	Ravine des cabris	300	Feb 91	53	18.6	24.5	1100
Clementine	Ravine des cabris	300	Feb 91	49	18.6	24.5	1100
Navel orange	Petite île	850	Apr 91	63	15.1	21.0	2500

sandwich (DAS) and two monoclonal antibodies (3CA5 and 3DF1, Ingenasa, Madrid, Spain) in indirect double antibody sandwich (IDAS). Plates were read on a Multiskan spectrophotometer. Samples with an optical density greater than twice that of the control for polyclonal antibodies or greater than three times that of the control for monoclonal antibodies were considered positive. The average of five negative controls was used in each plate as standard.

Aphid infestations. In the three groves located in Bassin Plat and Colimaçons, aphid infestation of young trees was recorded periodically by visual observation of the canopy. The genus or species of developing colonies was identified in the field using a handlens. Each tree was rated between 0 and 3 according to intensity of the infestation: 0 = no aphids, 1 = aphids present on less than three twigs, 2 = four to ten twigs infested, 3 = more than ten twigs infested.

Data analysis. Temporal spread of CTV was compared to three classical models (logistic, monomolecular and Gompertz) in their linearized form (4). Statitical evaluation of the models was performed with the STATITCF (ITCF, Paris) software. Spatial patterns of CTV infections, were analysed by doublets, ordinary runs, and 2DCLASS models as described by Campbell and Madden (4) and Nelson *et al.* (13). Clustering of *T. citricidus* colonies in the Mexican lime and clementine blocks were tested for spatial autocorrelation with the LCOR2 and 2DCLASS computer programm (6,13).

RESULTS

Increase and spread of CTV. The percentage of infected trees in the six orchards is presented in Table 2. The three groves at low to medium altitude (Mexican lime, combava and clementine) were entirely contaminated within 1.5 yr, whereas orchards planted at higher elevation had a much lower percentage of infected trees after the same period of time.

Data from three of the orchards with sufficient number of temporal assessments (Bassin Plat and Colimaçons) were fitted to logistic, monomolecular and Gompertz models (Table 3). The logistic model described adequately the observed data in the three orchards although the Gompertz model appeared better for clementine. Statistical analysis of the data demonstrated that the rate of CTV-disease increase in the lime orchard was approximately twice the rate found for sweet orange and clementine orchards.

Neither doublets, ordinary runs nor 2DCLASS analysis showed clustering of infected trees at any stage of infection for the six orchards monitored.

All the naturally aphid-infected trees in this survey were positive for the 3DF1 and the 3CA5 CTV epitopes simultaneously.

Aphid infestations. The relative abundance of aphid species on shoots

TABLE 2
PERCENTAGE OF CTV INFECTED TREES DETECTED BY ELISA IN THE SIX ORCHARDS.

Orchard	No. of trees	Alt. (m)	Months after planting			
			4	6	12	18
Mexican lime	90	150	17	26	79	99
Clementine	63	800	n.d.[z]	8	34	65
Valencia orange	54	800	n.d.	16	36	72
Combava	53	300	n.d.	94	100	100
Clementine	49	300	61	84	100	100
Navel orange	63	850	n.d.	n.d.	21	43

[z]n.d. = not determined

TABLE 3
COMPARISON OF THE RATE OF SPREAD OF CTV IN THREE CITRUS ORCHARDS WITH LOGISTIC, MONOMOLECULAR AND GOMPERTZ MODELS

Citrus species	Logistic model : $\ln[y/(1-y)]$[z]					
	R^2[y]	MSE[w]	Intercept	Rate[v]	SDR[u] (+/–)	D.W.[t]
Mexican lime	99.0*[x]	0.111	–3.49	0.437 a	0.0305	2.10
Clementine	99.1^{ns}	0.041	–3.87	0.253 b	0.0239	3.00
Valencia	99.1^{ns}	0.032	–3.03	0.217 b	0.0210	3.00
	Monomolecular model : $\ln[1/(1-y)]$[z]					
	R^2%	MSE	Intercept	Rate	SDR	D.W.
Mexican lime	93.3*	0.406	–1.43	0.307 a	0.0582	2.22
Clementine	97.0^{ns}	0.014	–0.44	0.078 b	0.0139	3.00
Valencia	92.2^{ns}	0.051	–0.47	0.092 b	0.0267	3.00
	Gompertz model : $-\ln[-\ln(y)]$[z]					
	R^2%	MSE	Intercept	Rate	SDR	D.W.
Mexican lime	96.7*	0.267	–2.37	0.363 a	0.0472	2.17
Clementine	99.9*	0.001	–1.81	0.146 b	0.0029	3.00
Valencia	96.7^{ns}	0.050	–1.56	0.143 b	0.0264	3.00

[z] y = incidence of diseased trees.
[y] R^2 = coefficient of determination.
[x] * = significant; ns = not significant at $P \leq 0.05$ (Fisher).
[w] MSE = Mean square error
[v] = rates with distinct letters are significantly different ($P \leq 0.05$).
[u] SDR = Standard deviation of rate
[t] D.W. = Durbin-Watson test for significance

is presented in Fig. 1. *Toxoptera citricidus* predominated (97% of colonies) throughout the year in the Mexican lime orchard, whereas *T. aurantii* Boyer de Fonscolombe, various *Aphis* spp. and *M. euphorbiae* Thomas were seldom encountered. Aphid species found in the Colimaçons orchards were much more diversified. Between July 1991 and October 1992, *T. citricidus* represented 53% of the colonies, *Aphis* spp. 28% and *M. euphorbiae* 19%. These percentages were similar in the clementine and the Valencia blocks, however the absolute number of infested shoots was about two fold higher in the former citrus species (542 and 239 colonies respectively). Colonies of *Aphis* sp. could not be clearly identified in the field, so these colonies were rated at the genus level. Examination under binocular microscope of some samples revealed the equal predominance of *A. spiraecola* Patch and *A. gossypii* Glover within this group. *M. euphorbiae* was frequently found in the Colimaçons blocks, although colonies developed poorly on the shoots.

The level of aggregation of trees infested with *T. citricidus* colonies was investigated for the Mexican lime orchard and the clementine block in Colimaçons. Among the 23 dates analysed for the Mexican lime orchard, only two had significant spatial autocorrelations (August 27, 1992, one adjacent tree within the row and September 14, 1992, one adjacent tree across the row). In the clementine orchard at Colimaçons, aggregation of infestation with *T. citricidus* was significant (2DCLASS) for six of the 12 dates tested, and revealed a preferential across the row dissemination (one to three adjacent trees).

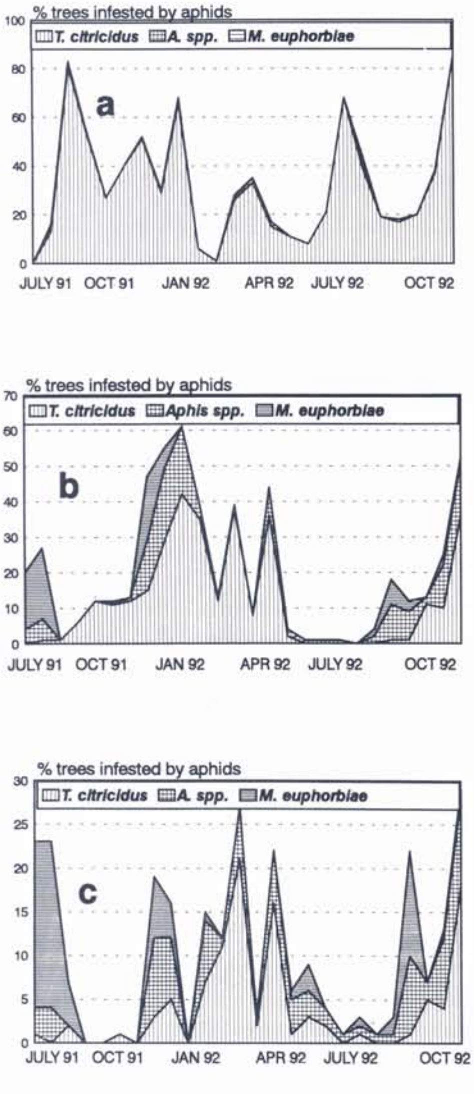

Fig. 1. Evolution of the percentage of trees infested by three aphid species in three citrus orchards. a - Mexican lime at Bassin Plat (150m), b - Clementine at Colimaçons (800m), c - Valencia orange at Colimaçons (800m).

DISCUSSION

Very rapid spread of CTV in the area where *T. citricidus* is present has been attributed to the high transmission efficiency of this vector, but dissemination was also associated with the planting of contaminated nursery material (2,8). In this survey of six new citrus plantings with CTV-free trees, infection occurred very quickly. Eighteen months after planting, 43 to 100% of trees were found infected. However, noticeable differences in the rate of disease spread were observed between the blocks. At low altitude, the Mexican lime, combava and clementine groves, showed a rapid rate of CTV infection. These orchards were planted in an area with warm climate and where citriculture is common. In contrast, the orchards established under the more temperate conditions of Colimaçons and Petite Ile, where citrus is restricted to a few small groves and backyard trees, CTV infection occurred at a considerably lower rate. No commercial citrus plantings were grown within 1 km from our experimental blocks. *Passiflora* spp. are the only known non rutaceous host for CTV (10,11,12). However, *Passiflora* is seldom cultivated in the island and it can therefore be assumed that citrus are the main source for contaminating aphids. The two different rates of infection might therefore have resulted from the amount and proximity of the surrounding inoculum. The random spatial pattern of infection observed in all plots suggests that the predominance of exotic CTV contamination is coming from outside the orchards.

However, low lying and high lying areas also differ in the relative abundance of vectors. *T. citricidus* is reported as the most efficient aphid vector of CTV (14) followed by *A. gossypii* and *A. spiraecola*. Predominance of *T. citricidus* and its continuous infestation pressure in the lime orchard of Bassin Plat could have contributed to the quicker spread of CTV in this lowland orchard also. Conversely, the limited abundance and frequency of *T. citricidus* at Colimaçons combined with the low density of infected trees surrounding the highland groves did not appreciably delay CTV infection as was expected.

To our knowledge, there is no evidence of *M. euphorbiae* as a vector for CTV, and variable transmission efficiencies of *Aphis* spp. were reported in certain citrus growing areas (14). Further experiments should be done to study the epidemiological role of these species in CTV spread. *T. aurantii* was very rarely identified during the survey. Given its low vectoring efficiency and assuming the semi-persistent transmision of the disease, this species is probably not significantly involved in the spread of CTV in Reunion.

The effect of citrus species in the transmission of tristeza by *A. gossypii*, has been recognized (10,16). In our study, similar disease development was observed in a pair of adjacent plots of distinct citrus species (clementine and combava at Ravine des Cabris, Valencia orange and clementine at Colimaçons).

Garnsey *et al.* (5) previously described the influence of orchard location on CTV spread in Hawaii. Our results present additional information on the influence of environmental factors on the dissemination of tristeza in the presence of *T. citricidus*.

The economic impact of lower production and severely affected trees induced by tristeza decreases as infection is delayed. Therefore, an evaluation of environmental factors that affect the spread of severe CTV strains is of great importance when setting up integrated control strategies against tristeza.

ACKNOWLEDGEMENTS

Authors are very thankfull to R. Hoareau and G. Sorres for facilitating the surveys in their citrus orchards. We also thank C. N. Roistacher for reviewing the manuscript.

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