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

M. Grisoni and C. Rivière

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 m elevation. Sweet orange and mandarin are the main cultivated citrus species. However, various species that are more susceptible to CTV, such as 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.

The presence of severe isolates of CTV limits the choice of rootstocks and strongly affects productivity of CTV-susceptible 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>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Altitude (m)</th>
<th>Planting date</th>
<th>No. of trees</th>
<th>Daily average temperature (C)</th>
<th>Annual rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexicanlime</td>
<td>Bassin Plant</td>
<td>150</td>
<td>Oct 90</td>
<td>90</td>
<td>19.6</td>
<td>1100</td>
</tr>
<tr>
<td>Clementine</td>
<td>Colimaçons</td>
<td>800</td>
<td>Jan 91</td>
<td>63</td>
<td>15.8</td>
<td>1450</td>
</tr>
<tr>
<td>Valencia</td>
<td>Colimaçons</td>
<td>800</td>
<td>Jan 91</td>
<td>54</td>
<td>15.8</td>
<td>1450</td>
</tr>
<tr>
<td>Cornbava</td>
<td>Ravinedescabris</td>
<td>300</td>
<td>Feb 91</td>
<td>53</td>
<td>18.6</td>
<td>1100</td>
</tr>
<tr>
<td>Clementine</td>
<td>Ravinedescabris</td>
<td>300</td>
<td>Feb 91</td>
<td>49</td>
<td>18.6</td>
<td>1100</td>
</tr>
<tr>
<td>Navelorange</td>
<td>Petiteile</td>
<td>850</td>
<td>Apr 91</td>
<td>63</td>
<td>15.1</td>
<td>2500</td>
</tr>
</tbody>
</table>
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 handheld lens. 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). Statistical 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 program (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.

<table>
<thead>
<tr>
<th>Orchard</th>
<th>No. of trees</th>
<th>Alt. (m)</th>
<th>Months after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Mexican lime</td>
<td>90</td>
<td>150</td>
<td>17</td>
</tr>
<tr>
<td>Clementine</td>
<td>63</td>
<td>800</td>
<td>n.d.²</td>
</tr>
<tr>
<td>Valencia orange</td>
<td>54</td>
<td>800</td>
<td>n.d.</td>
</tr>
<tr>
<td>Combava</td>
<td>53</td>
<td>300</td>
<td>n.d.</td>
</tr>
<tr>
<td>Clementine</td>
<td>49</td>
<td>300</td>
<td>61</td>
</tr>
<tr>
<td>Navel orange</td>
<td>63</td>
<td>850</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

²n.d. = not determined
### TABLE 3

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

<table>
<thead>
<tr>
<th>Citrus species</th>
<th>R²</th>
<th>MSE</th>
<th>Intercept</th>
<th>Rate</th>
<th>SDR</th>
<th>D.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Logistic model</strong>: ln[y/(1-y)]²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican lime</td>
<td>99.0*</td>
<td>0.111</td>
<td>-3.49</td>
<td>0.437a</td>
<td>0.0305</td>
<td>2.10</td>
</tr>
<tr>
<td>Clementine</td>
<td>99.1*</td>
<td>0.041</td>
<td>-3.87</td>
<td>0.253b</td>
<td>0.0239</td>
<td>3.00</td>
</tr>
<tr>
<td>Valencia</td>
<td>99.1*</td>
<td>0.032</td>
<td>-3.05</td>
<td>0.217b</td>
<td>0.0210</td>
<td>3.00</td>
</tr>
<tr>
<td><strong>Monomolecular model</strong>: ln[1/(1-y)]²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican lime</td>
<td>93.3*</td>
<td>0.406</td>
<td>-1.43</td>
<td>0.307a</td>
<td>0.0582</td>
<td>2.22</td>
</tr>
<tr>
<td>Clementine</td>
<td>97.0*</td>
<td>0.014</td>
<td>-0.44</td>
<td>0.078b</td>
<td>0.0139</td>
<td>3.00</td>
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<tr>
<td>Valencia</td>
<td>92.2*</td>
<td>0.051</td>
<td>-0.47</td>
<td>0.092b</td>
<td>0.0267</td>
<td>3.00</td>
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<tr>
<td><strong>Gompertz model</strong>: -ln[-ln(y)]²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican lime</td>
<td>96.7*</td>
<td>0.267</td>
<td>-2.37</td>
<td>0.563a</td>
<td>0.0472</td>
<td>2.17</td>
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<tr>
<td>Clementine</td>
<td>99.9*</td>
<td>0.001</td>
<td>-1.81</td>
<td>0.146b</td>
<td>0.0029</td>
<td>3.00</td>
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<tr>
<td>Valencia</td>
<td>96.7*</td>
<td>0.050</td>
<td>-1.56</td>
<td>0.143b</td>
<td>0.0264</td>
<td>3.00</td>
</tr>
</tbody>
</table>

* y = incidence of diseased trees.  
* R² = coefficient of determination.  
* * = significant; ns = not significant at P ≤ 0.05 (Fisher).  
* MSE = Mean square error  
* * = rates with distinct letters are significantly different (P ≤ 0.05).  
* SDR = Standard deviation of rate  
* 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. spiraeaeola* 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).
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. spiraeola*. 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

**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%
these species in CTV spread. *T. auran-
tii* was very rarely identified during
the survey. Given its low vectoring ef-
ficiency and assuming the semi-persis-
tent transmission of the disease, this
species is probably not significantly in-
volved in the spread of CTV in Reun-
ion.

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, Va-
lercia orange and clementine at Col-
imacons).

Garnsey et al. (5) previously de-
scribed the influence of orchard loca-
tion on CTV spread in Hawaii. Our re-
sults 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 pro-
duction and severely affected trees in-
duced 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 thankful to R. Hoareau and G. Sorres for facilitating the
surveys in their citrus orchards. We also thank C. N. Roistacher for re-
viewing the manuscript.

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