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# Relationship of citrus yield and virus infection in Trinidad

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## Abstract

Low yield is a serious problem of citrus in Trinidad but it is not known to what extent virus/viroid diseases contribute to yield reduction. This study is an attempt to quantify both the extent of infection of major virus/viroid diseases known to exist in citrus locally and the relationship of infection level with yield. The virus and virus-like diseases assessed in surveys were citrus tristeza virus (CTV), citrus exocortis viroid (CEV) and psorosis. The study began in 1996 and was conducted on Valencia orange, Ortanique tangor and Portugal mandarin established on sour orange rootstock. Techniques used in the survey included visual assessment of symptoms and both biological and serological indexing. In 1997, Ortanique had the highest level of CTV infection of 48.8% of trees, while in the other cultivars <10% tested positive. There were significantly fewer high yielding Ortanique CTV positive trees compared to CTV negative trees ($P = 0.042$). Fruit count of CTV positive trees was significantly lower than CTV negative trees in Valencia 2000 ($P = 0.004$) and Ortanique for a cumulative period of 1998 – 2000 ($P = 0.001$). All Ortanique trees and few trees of the other cultivars appeared infected with CEV. The yield pattern of infected trees over time did not suggest a reduction in yield associated with CEV infection. Valencia field 12069 had the most (51%) trees with psorosis-like bark symptoms. Presence of bark-scaling symptoms showed no relationship with yield. Of the three diseases studied, only CTV was responsible for yield reduction.

**Key words:** Citrus, Valencia, Ortanique, mandarin, virus and virus-like diseases, tristeza, exocortis, psorosis, yield

## Introduction

Reports of low yields in citrus in Trinidad over the years (Lucie-Smith, 1953; Ali *et al.,* 1973) and wide variation of tree yield within field suggested that all trees in the same location were not performing at full potential. This prompted an investigation of the various factors known to affect yield that were operating in the study fields. Mainly three virus diseases of citrus were known to exist in Trinidad.

Citrus tristeza virus (CTV), first reported to be present in Trinidad by Knorr (1967), was confirmed in 1991 (Aubert *et al.,* 1992), after the presence of the brown citrus aphid had become established. While there is clear correlation between yield and level of incidence of symptoms caused by severe strains of CTV (Sasaki, 1981) there has been continued concern about the non-lethal effects such as yield reduction (Cottin and Bourdeaut, 1992). Observation regarding bark symptoms of citrus exocortis viroid (CEV) on Rangpur lime in a rootstock trial was reported as early as 1963 (University of the West Indies, 1963). Severe strains of exocortis can cause bark scaling, stunting and reduced yield of trees with susceptible stocks (Davies and Albrigo, 1994).

Knorr (1967) had diagnosed the presence of psorosis in citrus trees in Trinidad from field symptoms during a visit in 1965. Field symptoms were detected at La Gloria estate in 1995 and this led to surveys that showed the condition to be widespread at both estates (Roistacher, 1996; Jones, 1998). Psorosis-A is slow-acting and tree deterioration results in trees being worthless within 10 – 20 years after lesions first appear (Wallace, 1978).

This study reports on a disease survey of three virus/viroid diseases in a citrus estate located in central Trinidad, with particular reference to its relationship with yield.

## Materials and methods

**Cultivars:** Study plots were located at the Caroni (1975) Limited Todds Road citrus estate in central Trinidad and the citrus types used were: orange (*Citrus sinensis* cv Valencia), mandarin (*C. reticulata* cv Portugal) and tangor (*C. sinensis* x *reticulata* cv Ortanique), all on sour orange rootstock.

**Yield estimation:** Yield levels were scored subjectively in 1997. This scoring was done only by three technicians, who were experienced in doing on-tree counts. Yield levels were therefore expected to conform to assigned count ranges. Mechanical hand-held counters were used from 1998 and counts were then converted in yield level categories. This was not done for Portugal since the major categories were easily distinguished. Scoring was generally done between January and March, before harvest, but was sometimes done in the previous year when fruit were mature but not at optimum stage for harvest. Yield estimation data were recorded in different sample sizes of trees (groups 1 and 2), of cultivars and fields (Table 1).

**Leaf sampling for CTV testing:** Young shoots of 1 - 2 weeks of age were normally used. Leaf petioles from slightly older shoots (3 - 5 weeks) were used as second choice material. Samples were taken around the tree at 1 - 1.5 m in height early in the morning, placed on ice in a plastic container and taken to the laboratory within 1 hour. The material was patted dry with tissue paper and the individual shoot or petiole cut in cross-section and the sap blotted four times on a nitrocellulose membrane. The stem was cut again and the exercise repeated. The blots were then tested

or stored at room temperature until testing by the Pathology Unit at the Caroni Research Station. The method used was direct tissue blot immunoassay (DTBIA) (Garnsey *et al.,* 1993).

**Table 1. Sample size for disease survey at Todds Road citrus estate**

| Cultivar/Field ID | Number of trees in survey | | |
|---|---|---|---|
| | Group 1[z] | Group 2 | Group 3 |
| Valencia 12069 | 188 | 39 | 15 |
| Valencia 12071 | 140 | 32 | 15 |
| Portugal 12050 | 142 | 30 | 15 |
| Portugal 12036 | 268 | 38 | 15 |
| Ortanique 12083 | 351 | 43 | 15 |

[z]The term 'group' refers to the sample size range used for data collection

Samples were collected annually in January to February from Ortanique (Field 12083) from 1997 to 2000. Valencia was sampled in 1997 and 2000 and the other survey fields in 1997 only. The period of sampling was selected because transmission and symptom expression (suggesting increased titre) were known to increase at lower temperatures (Lee *et al.,* 1994).

**Exocortis survey:** Budwood from the test trees was collected (four sticks per tree from around the canopy) for grafting onto indicator plants (Roistacher, 1991). These were Etrog citron on Volkamer lemon stocks or Etrog seedlings. The Etrog citron used was RMA 861, a selection imported for exocortis testing (Baksh, 1995). Blind buds from the test trees were budded onto four indicator plants per test tree. Knives were sterilized with 10% bleach solution between testing for each tree at budwood collection and at budding. Blind buds were inserted on the stock or directly on the Etrog if shoots were large. Shoots were cut to induce new growth if necessary. Plants were kept out in the open. Positives were identified by the occurence of mild or severe epinasty.

Testing began on September 25, 1997 with buds from the Ortanique plot. Valencia buds from Field 12069 were budded on October 2, 1997. Buds were untaped and scions cut back to encourage new growth after 4 weeks. The others were tested in March, 1998. Ortanique trees in a different location but from the same source were tested in July, 1998.

**Psorosis survey:** Trees in group 2 (Table 1) were examined visually for bark symptoms which were categorized as pustules or bark scaling symptomatic of psorosis-A. Symptoms of psorosis-B such as mottling and ringspots were not seen in the field and were not included in the survey. Confirmation of the disease was attempted by the Pathology Unit using biological indexing in a temperature controlled room but this was unsuccessful.

## Results and discussion

**CTV Survey:** The first test results in 1997 (Table 2) showed that only the Ortanique trees were heavily infected, the other cultivars not having adequate numbers of infected trees for yield comparison. Testing was concentrated on Ortanique and the Valencia fields were tested again in 2000. Valencia field 12071 showed negligible change in CTV status when retested in 2000.

**Table 2. Incidence of CTV as tested by immunological assay for citrus trees at Todds Road in February, 1997**

| Field | Cultivar | Number of trees | | | | |
|---|---|---|---|---|---|---|
| | | Total | Unsure | Positive | | Negative |
| | | | | Polyclonal IgG | Monoclonal MCA 13 | |
| 12083 | Ortanique | 43 | 2 | 21 | 14 | 19 |
| 12071 | Valencia | 50 | 1 | 4 | 6 | 43 |
| 12069 | Valencia | 44 | 0 | 4 | 4 | 39 |
| 12050 | Portugal | 31 | 0 | 0 | 0 | 31 |
| 12036 | Portugal | 34 | 0 | 0 | 0 | 34 |

Forty out of 96 Ortanique trees tested positive during the period 1997 to 2000. Data were analysed by yield level for 1997 to 2001 and by fruit count from 1998 to 2001. No statistical analysis was attempted beyond 1997 on mild-positive Ortanique trees since these trees eventually tested severe and were available in few numbers only. Even though the strain reacting to MCA13 antibody was labeled 'severe' no tree deaths from CTV were recorded in the study fields. Some tree death in grapefruit fields attributed to CTV occurred both at Todds Road and La Gloria estates. When additional trees were tested in 1998 those found negative were used for analysis of the 1997 data. As a result, a relationship of yield level and CTV (severe strain) presence was established $P = 0.042$ (Table 3).

**Table 3. Yield level and CTV infection status (2 x 2) contingency table for Ortanique tangor trees, 1997**

| Yield level | CTV (MCA13 positive) | | Total |
|---|---|---|---|
| | Present | Absent | |
| High | 2 | 30 | 32 |
| Low | 7 | 21 | 28 |
| Total | 9 | 51 | 60 |

Chi square statistic = 4.118 $P = 0.042$

This type of result was not repeated for the years 1998 to 2001. However, on-tree count data were used in comparing severe-infected trees (testing positive with MCA13) to non-infected trees in both Valencia and Ortanique (Tables 4 and 5).

Valencia showed significant differences in yield between severe positive and negative ($P = 0.004$) but no difference ($P = 0.725$) was seen between mild positives and negatives (Table 4).

**Table 4. Yield comparison of Valencia trees (Field 12069) testing monoclonal seropositive (severe strain) or polyclonal seronegative (mild and severe) in 2000**

| Test status | Number of trees + | Number[z] of trees - | Yield year | Mean yield of + trees | Mean yield of - trees | P value t-test |
|---|---|---|---|---|---|---|
| Severe | 8 | 18 | 2000 | 633 | 874 | 0.004 |
| Mild | 10 | 10 | 2000 | 473 | 516 | 0.725 |

[z] Trees from same rows as positives only

There was no significant yield difference between CTV(MCA13) positive and CTV negative trees of Ortanique on a year by year basis ($P > 0.05$), except for 2001, $P = 0.005$ (Table 5). When cumulative data for 1998 to 2001 were analysed no difference was seen $P > 0.05$ (Table 5). However, when trees which tested positive in 1997 were cumulatively compared to negative testing trees there was significant difference ($P < 0.05$) in every instance (Table 5). Non-cumulative testing showed significant difference ($P = 0.005$) in 2001 only (Table 5). The general trend suggests that the longer the interval after infection the more pronounced the effect on yield.

The yield reduction is in number of fruit unlike the reports by Sasaki (1981) and Rocha-Pena *et al.,* (1995) in which reduced fruit size contributed more to the yield reduction which was associated with stem pitting. Stem pitting was observed in Trinidad on West Indian lime by Aubert *et al.* (1992) and by one of the authors (R.P.), who also saw it on grapefruit, mandarin and sweet orange at Caroni during 1975. Limited orchards (data unpublished). But these were rare occurrences and not comparable to the Japanese experience (Sasaki, 1981).

**Table 5. Yield comparison of Ortanique trees testing monoclonal seropositive (severe strain) or polyclonal seronegative (mild and severe)**

| Number of trees | | First year | Yield & CTV- | Mean yield of | Mean yield of | *P* value t-test |
|---|---|---|---|---|---|---|
| + | - | CTV+ | year | + trees | - trees | |
| 13 | 30 | 1997 | 1998 | 160 | 209 | 0.187 |
| 13 | 47 | 1997 | 1999 | 188 | 249 | 0.073 |
| 13 | 47 | 1997 | 2000 | 244 | 261 | 0.563 |
| 13 | 27 | 1997 | 2001 | 40 | 126 | 0.005 |
| 26[z] | 77[z] | 1997 | 1998 & 1999 | 174 | 234 | 0.019 |
| 39[z] | 124[z] | 1997 | 1998 to 2000 | 197 | 244 | 0.02 |
| 52[z] | 151[z] | 1997 | 1998 to 2001 | 159 | 223 | 0.001 |
| 16 | 47 | 1999 | 2000 | 260 | 261 | 0.975 |
| 81[z] | 151[z] | 1997 to 2001 | 1998 to 2001 | 195 | 223 | 0.119 |

[z] cumulative data

In the year 2001, yield was poor and a major cause was likely to have been high populations of a new pest, Citrus Blackfly (*Aleurocanthus woglumi* Ashby) in the field (White, 2000). Perhaps this additional stress on CTV-infected trees emphasized the yield difference between the trees of differing CTV status.

**CEV survey**: Severe leaf epinasty was seen on indicator plants. There was some variation in the severity of symptom expression in test plants. This variation may be due to the presence of other citrus viroids that can also induce symptoms on citron (Roistacher, 1991). However, all 15 plants were considered positive for CEV in Ortanique, whereas all Valencia trees were negative in field 12071 (Table 6). An additional 12 Ortanique trees from another field were tested for CEV in July 1998. Eleven of these were considered positive, with one of uncertain status.

There were few positives in the other fields (Table 6) but yields of these trees continued to be variable (Table 7). This suggests that exocortis was not a major factor influencing yield variation within the fields under observation. This is not surprising as sour orange stock is considered not susceptible to exocortis (Ferguson and Garnsey, 1993).

**Table 6. CEV testing of citrus trees at Todds Road Estate, September 1997 - March 1998.**

| Cultivar / Field | Number of trees tested | | |
|---|---|---|---|
| | Total | Positive | Unsure |
| Portugal 12036 | 15 | 1 | 1 |
| Portugal 12050 | 15 | 2 | 1 |
| Valencia 12069 | 15 | 2 | 0 |
| Valencia 12071 | 15 | 0 | 0 |
| Ortanique 12083 | 15 | 15 | 0 |

**Psorosis:** Psorosis incidence based on bark symptoms in field trees was assessed in May 1998, since the exercise of developing indexing symptoms in the plant laboratory had failed thus far. This failure to elicit leaf symptoms and the sudden appearance and nature of field symptoms allows for the possibility that this disease may not be psorosis (Roistacher, 1996). Table 8 shows the results of this field survey. The survey was conducted on group 2 trees.

Yield of the trees with symptoms was compared (Table 9) to non-symptom trees but yields were not significantly different, suggesting that the 'putative psorosis condition' was not influencing yield variation. It is possible that yield differences would become pronounced at a later stage since psorosis-A decline is a slow process taking 10 – 20 years for full expression (Wallace, 1978).

**Table 7. Yield performance of CEV positive citrus trees at Todds Road**

| Field | Tree ID | Yield level by year | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 |
| 12036 | R14T1 | M | M | M | VL | na | na |
| | R14T9 | VH | VH | VH | VH | na | na |
| 12050 | R9T22 | L | H | VH | M | na | na |
| 12069 | R8T21[z] | na | na | L | L | L | VL |
| | R15T10 | na | M | H | M | H | na |

[z] also CTV infected; na – data not available

Yield levels: VH- very high; H-high; M- medium; L-low; VL- very low

**Table 8. Psorosis incidence based on bark symptoms at Todds Road Estate, 1998**

| Cultivar / Field | | Positive trees | | Negative trees | Total trees |
|---|---|---|---|---|---|
| | | Major lesions | Pustules | | |
| Portugal | 12036 | 7 | 0 | 25 | 32 |
| Portugal | 12050 | 1 | 0 | 29 | 30 |
| Valencia | 12069 | 12 | 8 | 19 | 39 |
| Valencia | 12071 | 0 | 0 | 33 | 33 |
| Ortanique | 12083 | 2 | 0 | 27 | 29 |

The difference in expression between the two Valencia fields was not due to age difference despite the fact that trees in 12071 were stunted compared to 12069. Both fields were ten years old and field symptoms resembling psorosis were first detected in field 12069 in 1996. In a subsequent survey Jones (1998) reported that the mean percentage of trees showing psorosis disease symptoms at Todds Road and La Gloria estates were 12.3 and 45.8%, respectively in oranges and 56.7 and 60.9%, respectively in grapefruit. Symptom expression may begin as early as ten years of age (Davies and Albrigo, 1994).

Symptoms are thought to be indicative of Psorosis A (less virulent than Psorosis B) but there has been no confirmation of the disease identification by laboratories in Florida (Citrus Research Center) and Italy (Instituto Agronomica Mediterraneo). Bark scaling symptoms in field 12036 were associated with branch and twig dieback and occupation by Azteca ant (*Azteca* spp.) colonies.

**Table 9. Comparison of mean yield of Valencia trees (Field 12069) with and without symptoms of psorosis expressed in 1998**

| Year | Mean yield of trees | | *P* value |
|---|---|---|---|
| | With symptoms | Without symptoms | |
| 1998 | 399 | 427 | 0.543 |
| 1999 | 448 | 483 | 0.513 |
| 2000 | 620 | 731 | 0.303 |
| 1998 – 2000 | 489 | 547 | 0.218 |

**Other observations**: During the psorosis survey in May, 1998 it was observed that 50% of the trees in field 12050 showed

longitudinal cracks in the bark of 5 - 10 cm diameter branches. Similar type bark cracks were observed in White Marsh grapefruit trees but the other Portugal field, 12036 had < 10% of trees with longitudinal bark cracks. Although these cracks can be associated with snow scale (*Unaspis citri* Comst.) there was no evidence of recent occupation by that pest. Vertical slits described by Polizzi *et al*. (1992) were associated with exocortis on trifoliate stock but not on sour orange.

These symptoms were seen on Portugal mandarin only and do not detract from our main conclusions. Citrus tristeza virus was associated with reduced yield of infected trees but was more of a problem in Ortanique than in Valencia during the study period. Citrus exocortis viroid was widespread in the Ortanique field only and not associated with yield variation. Psorosis-like symptoms were not associated with yield variation during the first four years after bark symptoms appeared.

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