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### Title

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# Natural Spread of Seedling Yellows and Sweet Orange and Grapefruit Stem Pitting Tristeza Viruses at the University of California, Riverside

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Natural spread of citrus tristeza virus (CTV) was first observed at the University of California, Riverside (UCR), in the 1950's, although various strains of CTV apparently had been introduced there in budwood and trees between 1917 and the 1930's (Wallace *et al.*, 1956; Roistacher *et al.*, 1979, 1980). Many sweet orange/sour orange trees affected by CTV at UCR were noted in the late 1950's. The vector, *Aphis gossypii* Glover, was relatively inefficient and each year approximately doubled the number of CTV-infected trees during the first few years after primary infestation of an orchard or locality (Dickson *et al.*, 1956). In Orange County, California, Nesbitt (1963) reported that about 8-12 years elapsed between the onset of the first tristeza symptoms in an orchard and obvious tristeza disease in 50 per cent of the susceptible trees. Calavan and Harjung (unpublished) annually indexed an orchard of 386 CTV-free Valencia orange trees planted at UCR in 1965, and found 50 per cent of the trees infected after 4 years. Calavan, in 1976 (unpublished), found 100 per cent of the trees in an adjacent navel orange orchard infected at 6 years of age. The rate of natural spread of CTV obviously had accelerated since the 1950's.

Numerous tests were made for citrus tristeza virus stem pitting (CTV-SP) of grapefruit and for seedling yellows tristeza (CTV-SY) in citrus from UCR and other California locations but, until recently, with two exceptions, these forms of the CTV complex were found only in trees derived or graft-inoculated from CTV-SP- or CTV-SY-infected trees.

Wallace (1957) reported CTV-SY in trees derived from several citrus importations. Martinez and Wallace (1964) showed that *A. gossypii* could readily transmit some isolates of CTV-SY from Valencia and navel orange to Mexican lime seedlings in the greenhouse. Later, Bar-Joseph and Loebenstein (1973) showed that *A. gossypii* easily transmitted CTV-SY in Israel. By 1977, Roistacher *et al.*, (1979) obtained conclusive evidence that CTV-SY and CTV-SP has spread naturally in citrus variety plantings in fields 7A, B and C at UCR (fig. 1). We immediately planned indexing of trees in various citrus orchards at UCR to determine the approximate extent and location of the natural spread. This paper reports the results of this investigation.

## EXPERIMENTS AND RESULTS

**Experiment 1.** Budwood collections from 132 grapefruit, lemon, sweet orange, and tangelo trees from 12 plots of different ages, located at various distances from known CTV-SY- or CTV-SP-infected trees in the variety plot (Roistacher *et al.*, 1979) were indexed on Duncan grapefruit, Eureka or Lisbon lemon, Madam Vinous sweet orange, Mexican lime, and Standard sour orange indicator seedlings. All trees indexed are believed to have been free of CTV when planted except, possibly, a few in fields 1B and 7A. Mexican lime indicator plants were observed periodically for CTV symptoms, and the other indicators were observed for CTV-SY symptoms. About 6 months after inoculation, the bark of the grapefruit and sweet orange indicators was peeled to detect CTV-SP symptoms.

TABLE 1  
 PRESENCE OF ORDINARY TRISTEZA VIRUS (CTV), SEEDLING YELLOWS TRISTEZA (CTV-SY), AND MODERATE-TO-SEVERE  
 GRAPEFRUIT OR SWEET ORANGE STEM-PITTING TRISTEZA (CTV-SP) IN CITRUS TREES  
 AT VARIOUS LOCATIONS IN UNIVERSITY OF CALIFORNIA, RIVERSIDE ORCHARDS, MAY-AUGUST 1978.

| Field  | Direction* | Avg.<br>distance†<br>(meters) | Age<br>(years) | Variety                 | Trees<br>indexed | Trees infected |        |        |
|--------|------------|-------------------------------|----------------|-------------------------|------------------|----------------|--------|--------|
|        |            |                               |                |                         |                  | CTV            | CTV-SY | CTV-SP |
| 7A‡    | —          | 0                             | 12             | Various<br>sweet orange | 6                | 1              | 5      | 0      |
| 12B    | 100°       | 160                           | 11             | Minneola<br>tangelo     | 26               | 10             | 13     | 7      |
| 16K    | 180°       | 175                           | 8              | Lisbon<br>lemon         | 10               | 0              | 0      | 0      |
| 11F    | 225°       | 435                           | 25             | Navel                   | 10               | 9              | 1      | 0      |
| 7E     | 270°       | 70                            | 7              | Valencia                | 10               | 9              | 1      | 0      |
| 6A     | 270°       | 350                           | 13             | Valencia                | 10               | 10             | 0      | 0      |
| 6B     | 270°       | 440                           | 8              | Navel                   | 10               | 10             | 0      | 0      |
| 6B     | 270°       | 440                           | 8              | Grapefruit              | 10               | 2              | 0      | 0      |
| 5C     | 280°       | 800                           | 12             | Valencia                | 10               | 9              | 0      | 0      |
| 1B     | 295°       | 875                           | 45             | Navel                   | 10               | 9              | 0      | 1      |
| 3B     | 320°       | 250                           | 8              | Lisbon<br>lemon         | 10               | 0              | 0      | 0      |
| 3B     | 325°       | 150                           | 8              | Navel                   | 10               | 2              | 0      | 0      |
| Totals |            |                               |                |                         | 132              | 71             | 20     | 8      |

\* 0° = north; 90° = east from field 7 variety plot.

† From nearest portion of variety plot.

‡ SE portion of the variety plot.

The results (table 1) indicated extensive spread of CTV-SY, and limited spread of CTV-SP for several hundred meters from the variety plot in the direction of the prevailing westerly wind (fig. 1). Most sweet orange and tangelo trees of bearing age indexed were found infected with CTV, CTV-SY, or CTV-SP, but few grapefruit and no lemon trees were found infected. Virus from seven trees caused moderate-to-severe stem pitting in grapefruit, but only one sweet orange indicator was severely pitted, stunted, and showed vein corking. Slightly pitted sweet orange and grapefruit seedlings were classified as having ordinary CTV or CTV-SY. In field 12B, the Minneola tangelo, infected with the CTV-SY which caused severe effects in sweet orange was severely stunted and pitted. Therefore, additional indexing was done in experiment 2 in areas near 12B, near the variety plots, and east of a planting in field 15H containing some Meyer lemon trees infected with SYTV.

**Experiment 2.** Indexing was done in October and November 1978 as in experiment 1, but without sour orange indicators. Budwood was collected from trees east, southeast, or south of known CTV-SY- or CTV-SP-infected trees. Except for some of the trees in field 7A, all trees tested are believed to have been CTV-free when planted.

Additional spread of CTV-SY and CTV-SP occurred within the Minneola tangelo planting in field 12B and into grapefruit and navel orange trees in field 12A to the east (table 2). Indexing was done on some groups of trees in fields 12C and 12D to the west of the Minneola tangelo block. Additional group indexing was done on numerous trees in field 16, to the east of several infected Meyer lemon trees in field 15H.

Virus transmitted from several of the trees indexed in our experiments, particularly from some of the naturally infected ones, caused severe stunting and yellowing of all varieties of indicator plants used, including sweet orange. Grapefruit CTV-SP was found in several sweet orange trees and, for the

first time at UCR, in a naturally infected grapefruit tree. Both experiments indicate abundant spread of CTV and CTV-SY to sweet orange and Minneola tangelo and significant natural spread of grapefruit and sweet orange CTV-SP despite the predominance of the other virus strains. In some plots to the east of known CTV-SY infected trees, the incidence of CTV-SY was found to equal or exceed that of the ordinary CTV believed present in most of the thousands of sweet orange, mandarin and tangelo trees at UCR.

## DISCUSSION AND CONCLUSIONS

The extensive spread of CTV-SY to the leeward side of the infested variety plot (tables 1 and 2) does not seem surprising, despite its apparent failure to spread naturally for many years after its introduction to UCR, when we consider the results of Martinez and Wallace (1964), Bar-Joseph and Loebenstein (1973), and Roistacher *et al.* (1979, 1980). We believe virus mutations are largely responsible, as suggested by Bar-Joseph (1978) and Roistacher *et al.* (1979, 1980).

Although CTV-SY originally was present in relatively few varieties imported as trees or budwood, its incidence now exceeds that of CTV in the southeast corner of the variety plot and in nearby Minneola tangelo trees to the ESE in field 12B. The predominance of CTV-SY in 12B contrasts sharply with the predominance of CTV noted by Calavan and Harjung (unpublished) in the late 1960's in field 6A and in our current sampling of sweet orange trees in fields 6A and 6B to the west of the variety plot (fig. 1). Our work confirms the efficiency of CTV-SY transmissions by *A. gossypii* (Roistacher *et al.*, 1980).

About half of the CTV-SY detected in sweet orange trees in the SE corner of field 7A was relatively mild and may be no more injurious than CTV commonly present in susceptible varieties. Only about 20 per cent of the CTV-SY found in field 12B was rated mild.

The CTV-SP of grapefruit and/or sweet orange was detected in relatively

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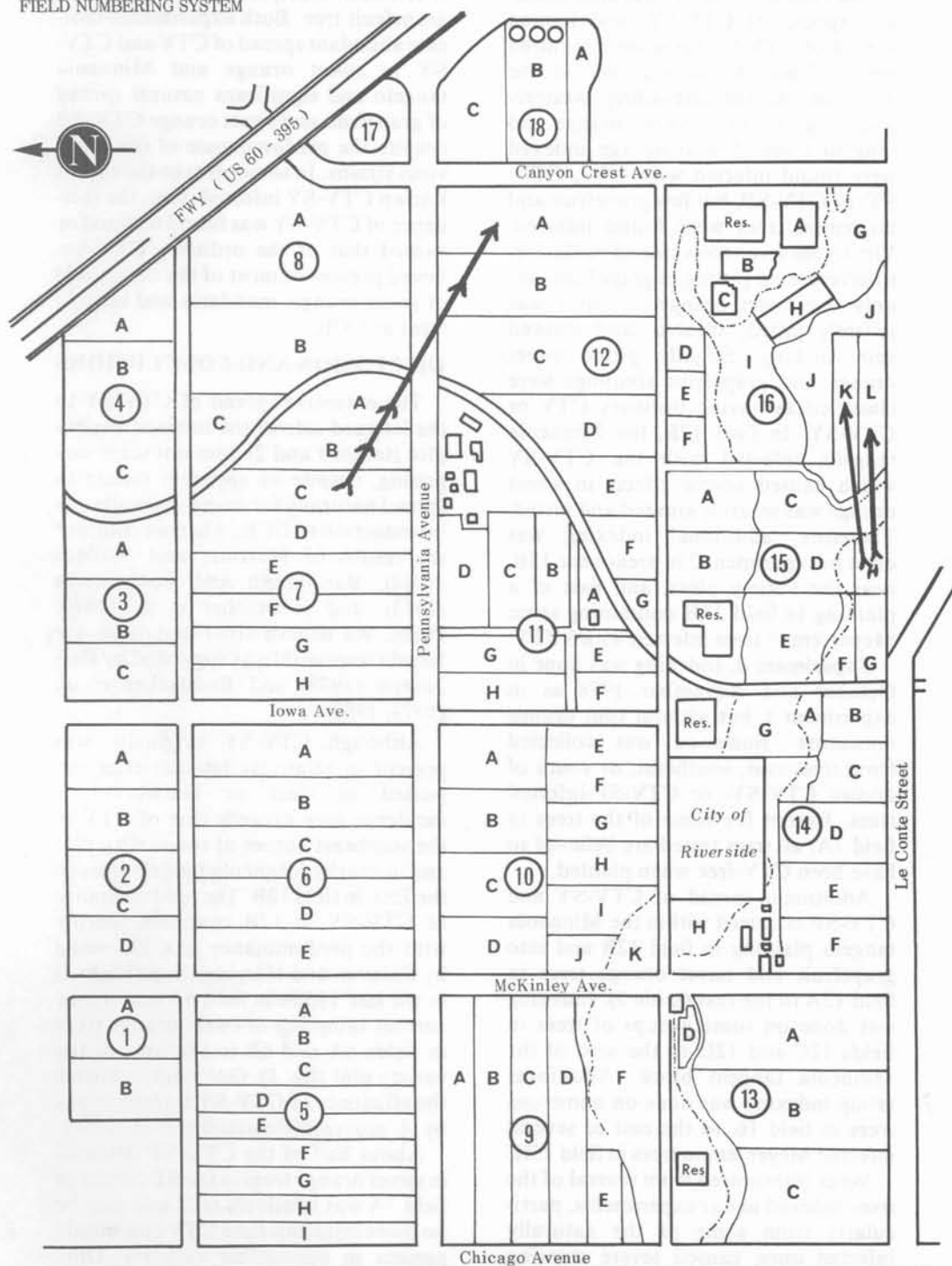


Fig. 1. Map of fields 1-18 at Citrus Research Center, Riverside. Arrows show principal movement of CTV-SY determined by indexing.

TABLE 2  
 OCCURRENCE OF TRISTEZA VIRUS (CTV) IN SELECTED TREES, MOSTLY IN THE VICINITY OF KNOWN INFECTIONS  
 OF SEEDLING YELLOWS (CTV-SY) AND MODERATE-TO-SEVERE GRAPEFRUIT OR SWEET ORANGE STEM PITTING  
 (CTV-SP) IN UNIVERSITY OF CALIFORNIA, RIVERSIDE ORCHARDS, OCTOBER-NOVEMBER 1978

| Field  | Direction* | Avg.<br>distance*<br>(meters) | Age<br>(years) | Variety                   | Single<br>trees<br>indexed | Groups<br>of trees<br>indexed† | Trees infected |        |        |
|--------|------------|-------------------------------|----------------|---------------------------|----------------------------|--------------------------------|----------------|--------|--------|
|        |            |                               |                |                           |                            |                                | CTV            | CTV-SY | CTV-SP |
| 7A     | E          | 20                            | 12             | Various<br>sweet orange   | 14                         | 0                              | 5              | 8      | 4      |
| 7D     | W          | 20                            | 8              | Sweet orange<br>and misc. | 15                         | 0                              | 4              | 4      | 0      |
| 8C     | ENE        | 100                           | 25             | Valencia                  | 10                         | 1                              | 7              | 4      | 0      |
| 12A    | ESE        | 280                           | 11             | Navel                     | 10                         | 0                              | 7              | 2      | 3‡     |
| 12A    | ESE        | 250                           | 11             | Grapefruit                | 0                          | 2                              | 1              | 1      | 1§     |
| 12B    | ESE        | 200                           | 11             | Minneola<br>tangelo       | 12                         | 0                              | 4              | 7      | 4      |
| 12C    | SSE        | 200                           | 11             | Valencia                  | 0                          | 1                              | 1              | 0      | 0      |
| 12D    | S          | 200                           | 10             | Navel                     | 0                          | 4                              | 4              | 0      | 0      |
| 15B    | S          | 375                           | 11             | Valencia                  | 0                          | 3                              | 3              | 0      | 0      |
| 15H    | E          | 10                            | 5              | Navel                     | 0                          | 5                              | 2              | 3      | 0      |
| 16K    | ENE        | 175                           | 8              | Valencia                  | 0                          | 10                             | 5              | 4      | 0      |
| 16K    | ENE        | 120                           | 8              | Grapefruit                | 0                          | 2                              | 0              | 0      | 0      |
| 16L    | E          | 150                           | 8              | Valencia                  | 0                          | 8                              | 4              | 4      | 0      |
| 16L    | E          | 130                           | 8              | Grapefruit                | 0                          | 2                              | 0              | 0      | 0      |
| 18C    | E          | 320                           | 11             | Lisbon<br>lemon           | 0                          | 2                              | 0              | 0      | 0      |
| Totals |            |                               |                |                           | 61                         | 40                             | 47             | 37     | 12     |

\* Distance and direction from variety block or, in fields 15H and 16K and L, from CTV-SY-infected Meyer lemon trees in 15H.

† Two to 5 trees per group on one set of indicators.

‡ One isolate caused pitting in grapefruit and sweet orange; the others in sweet orange only.

§ Pitting in grapefruit and sweet orange.

few trees, but was sometimes associated with severe stunting and stem pitting of indicator plants and orchard trees. As with CTV-SY, the CTV-SP isolates varied in severity, but appeared potentially the most dangerous ones detected.

There has been no indication of efficient natural transmission of any kind of tristeza virus to grapefruit in California, nor was any tristeza-virus infection detected in uninoculated Eureka or Lisbon lemon trees at UCR.

The mostly mild isolates of CTV-SY found in Cluster navel and Valencia trees in fields 15H and 16L, respectively, (table 2) were close to and directly east of some CTV-SY-infected Meyer lemon trees planted in 1973. The CTV-SY presumably moved in the direction of the prevailing westerly wind from these Meyer lemon trees (fig. 1).

Possible movement of CTV-SY and CTV-SP to the south from fields 7A, B, and C has not been adequately tested. There may have been a southward movement of about 100 meters in the Minneola tangelo trees in field 12B during the 11 years this plot has been planted. There is no nearby citrus north of fields 12A and B, and a former citrus variety plot in that location was removed prior to planting the recent plots of navel orange, grapefruit and tangelo trees in 12A and B.

The CTV-SY in old Valencia trees in field 8C may originate from fields 7B and C located to the WSW or, possibly, from a former variety plot which was located about 150 meters to the east prior to 1967. Cross protection by ordinary CTV in the old trees of field 8C was not necessarily complete and may have permitted some CTV-SY infection (Bar-Joseph, 1978).

Little westward movement of CTV-SY from the present variety plot was detected except in field 7D, which contains some CTV-SY-infected trees adjacent to the west border of the variety plot. The major spread of CTV-SY and CTV-SP appears to have been eastward (ENE to ESE) from major centers of infection.

The efficiency of group indexing of two to five trees (table 2) on a single indicator plant for the detection of CTV-SY or CTV-SP was not determined. It seems possible that severe symptoms of one isolate might be masked or modified by other CTV isolates. However, severe symptoms appeared in some group-indexed indicators.

There are no large commercial citrus orchards close to the eastern side of UCR and no large citrus areas within 5 km of UCR. It is possible, therefore, that natural spread of CTV-SY and CTV-SP from UCR has not reached large commercial plantings.

The effect on citrus trees of most commercial varieties by the various CTV-SY and CTV-SP isolates at UCR has not been determined, but severe damage has occurred on certain trees of some commercial varieties at UCR and on numerous trees of noncommercial varieties.

Because of the spread of CTV-SP of grapefruit and sweet orange, and because of the stunting and yellowing of sweet orange seedlings by certain severe isolates, UCR has undertaken a detection and eradication program, concentrating at first on the variety plot and the southeastern portion of the UCR orchard area. The CTV-SY- or CTV-SP-infected trees of the most valued selections in the variety plots will be repropagated and held in quarantine until freed of virus.

The tristeza-virus situation in nearby commercial orchards and in selected citrus areas of southern California will be examined in a cooperative project led by the California Department of Food and Agriculture. The detection of substantial numbers of commercial trees infected by severe CTV-SY or CTV-SP would serve to emphasize the need for accelerated research on cross protection by the use of mild strains (Cohen, 1976; Cox *et al.*, 1976; Muller and Costa, 1977; Thornton and Stubbs, 1976; Wallace and Drake, 1976).

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