# VIRUSLIKE DISEASES OF CITRUS

## Reaction of Young Citrus Trees to Graft Inoculations from Young Tree Decline and Sand Hill Decline-Affected Donors*

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Young tree decline (YTD) and sand hill decline (SHD), collectively referred to as YTD, are serious diseases of citrus trees on rough lemon rootstock in Florida. The former refers to the decline of young trees in new groves in flatwood areas, and the latter refers to the disorder affecting trees on sandy soils of the central ridge area.

Foliage symptoms associated with YTD have not been apparent in grove trees until they reach bearing age (5 to 7 years). Symptoms develop progressively with development of dull green foliage and upright growth of younger leaves. A nonpersistent chlorotic mottle similar to zinc deficiency patterns is often present and is a characteristic early symptom in some leaves during spring and fall. Canopy area is reduced and there is considerable twig dieback as well as loss of some secondary roots. Other symptoms of the YTD syndrome have been described (Feldman and Hanks, 1974*a*; 1974*b*).

Attempts to establish transmissibility of YTD have been inconclusive, therefore a series of extensive trials were started. Data are presented here for one such field trial initiated in the spring of 1972.

### MATERIALS AND METHODS

**Donor groves and source trees.** Eighty-six trees on rough lemon rootstock were selected from seven groves in the flatwood areas of east and south Florida and from three groves in the central ridge area (table 1). In each grove, except Grove I, four apparently healthy (all subsequently became YTD-affected within 2 years of initiation of experiment) and five early to midstage YTD-affected trees were selected as inoculum sources (table 1). Scion varieties of source trees were nucellar Valencia, and old-line Valencia, Pineapple, and Queen sweet oranges, and Red grapefruit (table 1).

**Inoculations for field trials.** Tissue from each source tree was used for inoculating three trees in each of two groups of young citrus trees on rough lemon rootstock (table 1). These trees were obtained from two sources. One group consisted of 298 presumed virus-free Hughes nucellar Valencia/rough lemon budlings of ca 0.5 cm trunk diameter. These were initially budded by L. C. Knorr and had been screenhouse-propagated (table 1). The other group consisted of 307 presumed virus-free trees (except possibly for tristeza) purchased from a local nursery (hereafter designated

*Florida Agricultural Experiment Stations Journal Series No. 6025.

TABLE 1
SOURCES OF INOCULUM FOR TRANSMISSION TRIALS TO BUDLINGS OF NUCELLAR VALENCIA AND TO A NURSERY SOURCE OF RED GRAPEFRUIT, PINEAPPLE, AND VALENCIA

| Donor grove and location | Scion var. | Age yrs. | No. source trees selected: Healthy* | No. source trees selected: Diseased | No. trees used for field trial: Nucellar val.: Inoc. | Nucellar val.: Not inoc. | Nursery source: Inoc. | Nursery source: Not inoc. | Scion var. |
|---|---|---|---|---|---|---|---|---|---|
| A. Flatwood (E)† | Red gpf. | 25 | 4 | 5 | 27 | 4 | 27 | 10 | R. gpf. |
| B. Flatwood (S)† | Pineapple | 12 | 4 | 5 | 27 | 4 | 27 | 5 | Pine. |
| C. Flatwood (S) | Pineapple | 10 | 4 | 5 | 27 | 4 | 27 | 5 | Pine. |
| D. Flatwood (E) | Queen | 13 | 4 | 5 | 27 | 4 | 27 | 5 | Val. |
| E. Flatwood (S) | Valencia | 12 | 4 | 5 | 27 | 4 | 27 | 4 | Val. |
| F. Flatwood (S) | Valencia | 10 | 4 | 5 | 27 | 4 | 27 | 4 | Val. |
| G. Flatwood (E) | Nucellar Valencia | 11 | 4 | 5 | 27 | 4 | 27 | 4 | Val. |
| H. Ridge | Valencia | 40 | 4 | 5 | 27 | 4 | 27 | 4 | Val. |
| I. Ridge | Valencia | 20 | 2 | 3 | 15 | 4 | 15 | 4 | Val. |
| J. Ridge | Valencia | 25 | 4 | 5 | 27 | 4 | 27 | 4 | Val. |
| | Totals | | 38 | 48 | 258 | 40 | 258 | 49 | |

*All "healthy" source trees exhibited YTD symptoms within 3 to 24 months after initiation of transmission experiment.

†E = east coast areas; S = south-central areas.

as nursery trees) and included scion varieties of Red grapefruit, Pineapple, and Valencia (table 1). Trunk diameter of the nursery trees varied from 2.5 cm for the Red grapefruit, to 1.5 to 2.0 cm for the Valencia and Pineapple, respectively.

Nucellar Valencia trees were graft inoculated in early March 1972 and were planted 3 weeks later at the Agricultural Research and Education Center in Lake Alfred using a 5 x 6 m spacing. Nursery trees were planted at the same spacing in an area continguous to the above in mid-April and were graft inoculated 3 weeks later. Inoculum consisted of two buds plus a 0.5 x 2 cm root-bark patch (from one half of the source trees). Grafts were placed ca 15 cm above the bud union. All knives and pruning shears were disinfected prior to collection of tissue from each source tree and prior to inoculation of each budling (Garnsey and Jones, 1967). Groups of 4 to 10 noninoculated controls (table 1) were planted within each group of trees inoculated from a given donor grove. All trees were maintained according to recommended commercial grove practices. To avoid the possibility of minor element deficiencies, two sprays containing B, Mo, Cu, Fe, Zn, and Mn were applied each year.

As a precaution against transmission from other sources, an additional group of 20 nucellar Valencias and 44 nursery trees, inoculated and controls, were planted in 55 x 55 cm tubs and are currently maintained in a screenhouse.

Data are given only for the field-planted trees. Assessment of zinc deficiency-like symptoms were made during winter and early spring 1974-1975 and tree canopy measurements (height x width) were made in March 1975. Data were analyzed by the modified Student's "t" test (Snedecor and Cochran, 1967) and are presented as mean canopy size ($m^2$) for noninoculated controls and for inoculations from healthy as well as from diseased source trees that elicited, in inoculated trees, a reduction in canopy size of greater than 20 per cent.

## RESULTS

**Nucellar Valencias**. Significant ($P = 0.05$) reductions in canopy size (stunting) were observed following inoculations from both healthy and diseased source trees from all six Valencia donor groves (table 2). Stunting was observed only in inoculations from healthy source trees from Groves D (Queen) and C (Pineapple) and only from diseased source trees from Grove A (Red grapefruit).

Reduction in canopy was generally in the range of 10 to 30 per cent although greater reductions were occasionally observed. Transmission of the stunting factor was inconsistent in severity as well as in distribution from source trees in any donor grove (table 2). Often one and occasionally two of the three Hughes nucellar replicates inoculated from a given donor would be moderately or severely stunted, while the other(s) would exhibit slight or no stunting. In a few instances, all three replicates exhibited similar stunting.

Moderate to severe stunting was observed in ca 23 per cent of the nucellar Valencias (table 2) in inoculations from 21 healthy and 23 diseased source trees (ca 50 per cent of each source group). In general, donor groves that elicited the most stunting also had more source trees responsible for stunting (table 2).

A zinc-like deficiency symptom, only in the inoculated trees, was observed on some leaves in seven trees (table 2) in observations made in December 1974 but no symptoms were observed in these same trees 5 months later.

Root-bark grafts did not appear to enhance transmissibility of the incitant(s) responsible for reduction in canopy size or for production of the zinc-like deficiency symptom (data not shown).

**Nursery trees.** Significant reductions in canopy were evident in nursery Valencias inoculated from both healthy and diseased source trees from two Valencia groves (I and J) and only from diseased source trees from two other Valencia groves (E and G) (table 2). Similar canopy reductions were noted in nursery Pineapple trees inoculated from both

TABLE 2
RESPONSES EXHIBITED BY NUCELLAR VALENCIA AND NURSERY SOURCE TREES AS EXPRESSED BY THE DEVELOPMENT OF A ZINC-LIKE DEFICIENCY SYMPTOM AND BY A REDUCTION IN CANOPY SIZE FOLLOWING GRAFT INOCULATIONS FROM DISEASED AND HEALTHY TREES IN YTD-AFFECTED GROVES

| Donor groves | Avg. canopy size ($m^2$) noninoc. controls | Avg. canopy size ($m^2$) in inoculations from source trees rated | | No. inoculated trees with Zn deficiency symptoms† | No. buildings with No. buildings with > 20% canopy reduction/ no. of source trees rated | |
|---|---|---|---|---|---|---|
| | | Healthy* | Diseased | | Healthy* | Diseased |
| | | **Nucellar Valencia** | | | | |
| A. (Red gpf.) | 6.4 a‡ | 5.9 ab | 5.3 b | 0 | 0 | 4/4 |
| B. (Pine.) | 6.4 ab | 7.2 a | 5.7 ab | 0 | 0 | 2/2 |
| C. (Pine.) | 6.4 a | 5.2 b | 6.5 a | 0 | 4/2 | 0 |
| D. (Queen) | 6.4 a | 5.2 b | 6.1 a | 1 | 3/2 | 0 |
| E. (Val.) | 6.4 a | 4.9 b | 5.2 b | 0 | 4/4 | 5/4 |
| F. (Val.) | 6.4 a | 5.8 b | 5.6 b | 1 | 1/1 | 3/2 |
| G. (Val.) | 6.4 a | 5.0 b | 5.7 b | 2 | 6/4 | 5/3 |
| H. (Val.) | 6.4 a | 4.7 b | 5.5 b | 1 | 4/4 | 5/3 |
| I. (Val.) | 6.4 a | 5.4 b | 4.4 c | 2 | 3/2 | 6/3 |
| J. (Val.) | 6.4 a | 5.4 b | 5.5 b | 0 | 3/2 | 2/2 |
| | | | Totals | 7 | 28/21 | 32/23 |
| | | **Nursery source** | | | | |
| A. (Red gpf.) | 11.1 a | 10.5 a | 10.9 a | 0 | 0 | 0 |
| B. (Pine.) | 9.4 a | 8.1 a | 8.4 a | 4 | 0 | 0 |
| C. (Pine.) | 9.4 a | 5.9 b | 6.1 b | 0 | 7/4 | 7/4 |
| D. (Queen) | 9.2 a | 9.7 a | 8.7 a | 0 | 0 | 0 |
| E. (Val.) | 9.2 a | 9.7 a | 7.6 b | 5 | 0 | 4/2 |
| F. (Val.) | 9.2 a | 8.3 a | 9.0 a | 5 | 1/1 | 1/1 |
| G. (Val.) | 9.2 a | 9.4 a | 7.9 b | 1 | 0 | 2/2 |
| H. (Val.) | 9.2 a | 8.2 a | 8.7 a | 4 | 0 | 0 |
| I. (Val.) | 9.2 a | 6.6 b | 6.9 b | 2 | 5/2 | 5/3 |
| J. (Val.) | 9.2 a | 7.4 b | 7.7 b | 3 | 5/4 | 4/2 |
| | | | Totals | 24 | 18/11 | 22/14 |

*All "healthy" source trees exhibited YTD symptoms within 3 to 24 months after initiation of transmission experiment.
†No Zn deficiency symptoms appeared in noninoculated control trees.
‡Values with a letter in common are not significantly different (P = 0.05).

healthy and diseased sources from Pineapple Grove C. Stunting was not observed in trees inoculated from any of the source trees from Groves A (Red grapefruit), B (Pineapple), D (Queen), and H (Valencia) (table 2). Donor groves that elicited the most stunting, although inconsistently transmitted, also had the greatest number of source trees responsible for the stunting. The agent(s) responsible for the zinc-like deficiency symptom, observed only in the inoculated trees, was essentially equally transmitted from healthy and diseased source trees. This symptom also did not appear to be consistently associated with severity of stunting (table 2).

Moderate to severe stunting was observed in ca 16 per cent of the nursery trees (table 2) in inoculations from 11 healthy and 14 diseased source trees (ca 30 per cent of each source group). Root-bark grafts did not increase transmission of the incitant(s) responsible for reduction in canopy size or for production of the zinc-like deficiency symptom (data not shown).

## DISCUSSION AND CONCLUSIONS

Citrus is well known for irregularities in transmission of some incitants (McClean and Oberholzer, 1965; Calavan *et al.*, 1968). Consequently, it is not too surprising that the incitant(s) of certain symptoms (stunting and zinc-like deficiency) of the YTD syndrome would also be inconsistently transmitted.

Transmission of the stunting factor to nucellar Valencias, although differing in virulence, appeared to be quite consistent in inoculations from all six Valencia donor groves. Transmission, however, was less consistent from the other donor groves in that either healthy or diseased source trees from the Queen, Pineapple sweet orange, and Red grapefruit donor groves elicited stunting in the nucellar Valencias. Transmission of the stunting factor to nursery trees was even more inconsistent. In the latter case, the stunting factor was transmitted from healthy and diseased-source trees from only two Valencia and one Pineapple donor groves while transmission was obtained only from the diseased source trees from two other Valencia donor groves.

Unequal transmission of the stunting factor from healthy and diseased source trees within a given donor grove is difficult to explain. Transmission of the stunting factor does not appear to be related to early stage of YTD involvement in the source tree in a manner similar to transmission of the Madam Vinous stem pitting factor (Feldman and Hanks, 1974*a*; 1974*b*), nor does there appear to be improved transmission of the stunting factor during leaf flush. If transmission is related to a lack of visible symptoms in the source trees, we would expect a high incidence of transmission from source trees rated "healthy", since these trees subsequently exhibited YTD within 3 to 24 months after collection of budwood. A leaf flush-associated increase in transmission of the stunting factor would have elicited a higher incidence of transmission from the healthy source trees, which were in leaf flush at time of inoculation to the nucellar Valencia, and a higher incidence of transmission from the diseased source trees, which were in flush at the time of inoculation to the nursery trees. These associations were not observed.

The greater number of source trees that elicited stunting in the nucellar Valencias as compared with those in the nursery trees may be accounted for, in part, by the difference in time of inoculation. The former were inoculated in early March when day temperatures were 22-27° C with nights at 13-18°C, while the latter were inoculated in early May when day temperatures were 32-35° C with nights at 23-26°C. The effect of temperature, as has been observed on transmission and symptom development of other citrus diseases (Schwarz, 1970; Bar-Joseph and Loebenstein, 1973; and Roistacher *et al.*, 1974), could be a factor affecting titre and distribution of the stunting agent in the donor and could account for the more consistent transmission of the stunting factor to nucellar Valencias during cooler weather. Although more zinc-like deficiency symptoms were observed in nursery trees, this symptom did not appear to be consistently associated with stunting. Leaves with zinc-like deficiency symptoms were generally smaller and tended to exhibit an upright growth habit characteristic of leaves of YTD-affected trees.

Another factor that could account for failure of nursery trees to consistently exhibit stunting, when inoculations from the same source trees elicited stunting in nucellar Valencias, would be the need for a longer incubation. This increase in incubation may be required possibly because nursery trees were carrying a mild form of the stunting factor prior to inoculation, and only inoculations with the more aggressive strains of the stunting factor would more readily affect canopy development.

Identity of the stunting factor is currently not known. All source trees were found to carry tristeza virus. Many also carried exocortis and a stem pitting factor for Madam Vinous, Pineapple, and grapefruit (Feldman and Hanks, 1974*a*). In addition, nursery trees may be carrying the tristeza virus. Indexing for the above stem pitting factors and for tristeza are currently in progress. Similar indexing is also being done on the nucellar Valencias.

Rough lemon rootstocks in Florida have always shown a high degree of tolerance to tristeza so that a transmissible factor affecting trees on this stock poses a consideration for either a new transmissible factor, a specialized strain of tristeza, or a combination of the two (Feldman and Hanks, 1974*a*; 1974*b*).

These data provide evidence that the incitant(s) of some of the symptoms (zinc-like deficiency and stunting) of the YTD syndrome is transmitted, though inconsistently, and that these symptoms appear to require an incubation of more than 2 years. Several more years may be required for the fruiting-associated stress to develop in order to establish whether or not the entire YTD syndrome will be reproduced.

## ACKNOWLEDGEMENT

The authors are indebted to O.T. Kirkland, C.C. Rafferty, N.F. Prather, W.J. Alexander, K. Eisenschenk, and B.R. Beasley for their excellent technical assistance and to Dr. B.S. Buslig, G.J. Edwards, and S.R. Wells for statistical analyses of these data.

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