Even though only a few trees were chosen for this study, many samples of root and leaf water potential, and stomatal diffusion resistance were taken. These samples showed consistent diurnal differences in water relations between blight or YTD trees and healthy trees.

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YOUNG TREE DECLINE AND SAND HILL DECLINE; STATUS OF INDEXING INVESTIGATIONS

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Abstract. **One hundred nineteen trees (donors) from 15 groves were indexed on 12 Citrus spp. (indicators) to determine virus content of trees affected with young tree decline (YTD) and sand hill decline (SHD). Indexing procedures and selection of indicators were designed to ascertain presence of at least 18 known citrus virus and virus-like diseases. Data from 1972 and 1973 indexing revealed primarily 3 transmissible agents: exocortis, tristeza, and a previously unreported stem pitting factor in seedlings of Mme. Vinous and Pineapple sweet orange. Stem pitting in these sweet orange indicators as well as in Rusk citrange, grapefruit, and Citrus excelsa appears to be dormancy/temperature related. Dual infections of tristeza and the Mme. Vinous-Pineapple stem pitting factor were present in all grove locations and there was a good correlation with YTD and SHD when budwood for indexing was obtained from donor trees in the early stage of disease. Although very extensive transmission and propagation experiments are in progress, neither transmission nor propagation of YTD and SHD has yet been demonstrated.**

Florida Agricultural Experiment Stations Journal Series No. 5545. We thank S. M. Garnsey for supplying some of the citrus viruses used in these experiments. We are indebted to C. C. Rafferty, O. T. Kirkland, N. F. Prather, K. Eisenschenk, W. J. Alexander, B. R. Beasley, and E. K. King for their excellent technical assistance.

Young tree decline (YTD) and sand hill decline (SHD) are new and serious diseases of citrus trees on rough lemon rootstocks *(Citrus jambhiri)* in Florida. The former refers to decline of young trees in new groves in the flatwood areas, and the latter refers to the disorder affecting trees on sandy soils of the central ridge area. Both YTD and SHD (hereafter referred to as YTD) are considered to be diseases of the stock because the rough lemon root system appears to be the primary stressed area (9). Loss of trees from YTD, now estimated at 50,000 to 75,000 acres, has been most prevalent among groves of sweet orange on rough lemon rootstock. Grapefruit *(C. paradisi)* on rough lemon has also been severely affected in some areas. Some groves have become commercially unproductive in 6 years (5).

Etiology of YTD currently is unknown. Mineral nutrition (1), nematodes (13), lead toxicity from air pollution (Koo and Feldman, unpublished), fungus (DuCharme, unpublished), or mycoplasma-like agents (12) do not appear to be responsible for this decline.

Symptoms develop progressively with development of dull green foliage and upright growth of younger leaves. A chlorotic mottle similar to zinc deficiency patterns is a characteristic early symptom and is present in some leaves during spring and/or late fall. Bloom is initially profuse, but in the later stages of decline it is erratic and sparse. Leaves are smaller and fewer in the subsequent and often delayed flushes, and there is considerable twig dieback as well as loss of some secondary roots. In some instances, the entire tree may suddenly wilt and collapse. Small patches of gum are often found randomly distributed in the stock bark at the bud union. Conical pegs are also present in the stock bark with corresponding pits in the wood near the bud union (Fig. 1).

Patterns of distribution of affected trees and subsequent spread to other trees in affected groves (5) indicate that YTD may be an insect-vectored disease. Experiments on transmission of YTD by bud, side graft, or bark patch grafts to budlings and older field trees are in progress, as are experiments to reproduce YTD by propagation.

These investigations, initiated in 1972, were undertaken to determine the virus content of both YTD- and SHD-affected trees on rough lemon rootstocks (6). Indexing procedures and selection of indicators were designed to ascertain presence of at least 18 known citrus virus and virus-like diseases.

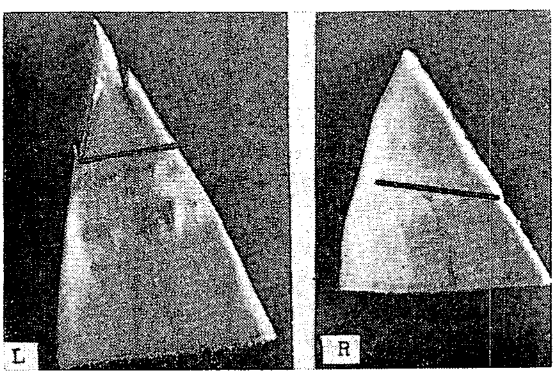

Fig. 1. Bark patch at stock-scion area of 14-year-old Valencia orange tree showing conical peg formation in stock bark just below bud union from tree affected with young tree decline (L). Corresponding pits are found in the wood. Bark patch from healthy tree (R). Line indicates stock-scion union.

Materials and Methods

One hundred nineteen trees on rough lemon rootstock were selected from 9 groves located in the flatwood areas of east and south Florida and from 6 groves in the central ridge area. Scion varieties of source trees were Valencia, as well as nucellar Valencia, Pineapple, Hamlin, Queen, and Red grapefruit (Table 1). Four or 5 of apparently healthy as well as of YTD-affected trees were selected in each grove. Unfortunately, many of the donor trees initially rated "healthy" began to exhibit decline symptoms either at the time of budwood collection or within 3 to 24 months. Trees rated "0" were considered "healthy;" those rated "1/2" (early disease) had occasional clusters of leaves exhibiting zinc deficiency-like patterns; trees rated "1" were in mid-stages of disease; and trees rated "2" or "3" were in later stages of decline. Tree condition was assessed at time of budwood collection and every 2 or 3 months thereafter. All knives and pruning shears were disinfected prior to collection of budwood from each donor tree and prior to inoculation of each indicator seedling (7).

Indicator seedlings. 'Eureka' lemon *(C. limon)*, *C. excelsa*, 'Etrog' citron *(C. medica)* clone OES-2, 'Mexican' lime *(C. aurantifolia)*, sour orange *(C. aurantium)*, rough lemon, 'Orlando' tangelo *(C. reticulata* X *C. paradisi)*, 'Mme. Vinous' sweet orange *(C. sinensis)*, 'Pineapple' sweet orange, 'Duncan' grapefruit, 'Rusk' citrange *(C. sinensis* X *Poncirus trifoliata)*, and 'Dweet' tangor *(C. reticulata* X *C. sinensis)*. Propagation of the seedlings, general plant maintenance, and inoculation procedures have been published (6). Because of limitations in screenhouse space, it was necessary to limit the selection of indicators for indexing some of the donor trees. Inoculations were also made with known individual virus isolates or mixtures and included: a moderate reacting isolate of tristeza (T_1M) that was generally more aggressive than isolates found in YTD-affected trees; a severe reacting isolate of tristeza (T_3A); exocortis; psorosis; xyloporosis; infectious variegation; crinkly leaf; and an isolate of Milam stem pitting. After inoculations, indicator seedlings were maintained in an insect-proof screenhouse. Orlando tangelo bud-inoculated plants were later transferred to field plots.

Results

Symptoms noted in the inoculated indicator seedlings are listed below.

Mexican lime. Symptoms of generally mild tristeza (vein clearing and light to moderate stem pitting) were observed in inoculations from 100 of 102 healthy and YTD-affected trees from all 14 groves tested (Table 1). Tree condition at time of budwood collection appeared to have little influence on transmissibility of tristeza virus (Table 2).

Etrog citron. Seedlings inoculated with buds from healthy as well as YTD-affected trees from 12 of 14 groves showed typical symptoms of exocortis (epinasty, vein corking, and downward curling of leaves) from 68 of 103 donors (Table 1). Tristeza symptoms (leaf or vein yellowing and mild to moderate stem pitting) were also noted. Tree condition at time of budwood collection also did not appear to have any influence on transmissibility of the exocortis virus (Table 2).

Sour orange. Most seedlings exhibited no foliage symptoms. A slight reduction in growth as well as a nonpersistent chlorotic mottle was observed in many of the seedlings inoculated from donor trees in groves B, L, M, and N (Table 1). Stem pitting of sour orange, believed caused by tristeza virus (8), was transmitted from 51 of 102 healthy as well as YTD-affected trees from 13 of 14 groves. No stem pitting was observed in grove K, although budwood from these trees produced typical tristeza symptoms in Mexican lime (Table 1). Tree condition at time of budwood collection also did not have any appreciable influence on transmission of the stem pitting agent to sour orange (Table 2).

Mme. Vinous. Only a few seedlings exhibited a slight to moderate chlorotic mottle. Stem pitting, mostly light to moderate in Mme. Vinous, was observed only in shoots from overwintered-late fall, and spring flush and was transmitted from all 15 groves in inoculations from 85 of 119 healthy as well as YTD-affected trees (Table 1). These 85 donor trees also carried tristeza virus. No stem pitting was noted in Mme. Vinous seedlings inoculated with the relatively moderate T_1M tristeza isolate. This isolate was generally more aggressive in Mexican lime than were isolates from most of the donor trees. Several small pits were observed in 1 of 6 Mme. Vinous seedlings inoculated with the severe T_3A tristeza isolate. Mme. Vinous stem pitting was readily transmitted to other Mme. Vinous seedlings and there was no remission of stem pitting symptoms after seedlings were severely cut back and allowed to grow. Unlike tristeza and exocortis virus, transmission of stem pitting to Mme. Vinous was considerably less as YTD-induced stress increased (Table 2).

Stem pitting was transmitted from 20 of 23 donor trees rated "1/2" (Table 2), representing 12 groves. Stem pitting was transmitted from 21 of the 33 donor trees rated "0" (apparently healthy). Nineteen of these 21 donor trees, carrying the stem pitting factor, exhibited YTD symptoms in 3 to 21 months after budwood collection;

Table 1. Symptoms or virus in indicator plants bud inoculated from presumed healthy and young tree decline (YTD)-affected citrus trees.[z] Summary 1972 and 1973 indexing data.

Grove and location	Scion variety	Age (yr)	Indicator plant with virus or symptom noted: Mme. Vinous (stem pitting)	Pineapple (stem[y] pitting)	Citrus excelsa (stem[y] pitting)	Sour orange (stem pitting)	Duncan grapefruit (stem[y] pitting)	Rusk citrange (stem[y] pitting)	Mexican lime (tristeza)	Citron (exocortis)
A. Ridge	Valencia	25	8/12[x]	3/6	4/5	6/9	2/4	4/4	8/8	9/9
B. Ridge	Valencia	40	4/4	3/4	4/4	9/10	2/4	4/4	4/4	4/4
C. Ridge	Valencia	14	1/5	--	--	1/3	--	--	3/3	2/2
D. Ridge	Valencia	18	3/5	--	--	1/2	--	--	2/2	2/2
E. Ridge	Valencia	20	5/7	--	--	2/3	--	--	3/3	3/3
F. Flatwood(S)[w]	Valencia	12	9/10	6/6	4/4	3/8	3/4	2/4	10/10	4/10
G. Flatwood(S)	Valencia	10	8/8	5/6	5/5	2/7	4/4	2/4	10/10	10/10
H. Flatwood(S)	Valencia	18	2/4	--	--	--	--	--	--	--
I. Flatwood(E)[v]	Valencia	11	7/10	3/6	4/4	2/10	2/4	2/4	9/10	10/10
J. Flatwood(E)	Nuc. Val.	11	2/6	4/4	5/5	2/6	3/4	4/4	6/6	0/6
K. Flatwood(E)	Red grft.	25	3/9	1/6	0/6	0/10	0/4	3/6	5/6	6/6
L. Flatwood(E)	Queen	13	8/9	5/6	4/4	3/8	4/4	3/4	10/10	0/12
M. Flatwood(S)	Pineapple	12	8/10	6/6	4/4	4/8	5/5	2/4	10/10	5/9
N. Flatwood(S)	Pineapple	10	9/10	5/6	5/5	6/8	4/5	4/4	10/10	3/10
O. Ridge	Hamlin	12	8/10	5/6	5/6	9/10	2/5	2/6	10/10	10/10
Totals			85/119	46/62	44/52	50/102	31/47	32/48	100/102	68/103

[z]Both young tree decline (YTD) and sand hill decline (SHD) are included in the designation of "YTD."

[y]From 1973 indexing data.

[x]Number of donor trees eliciting the specific response/total donor trees indexed.

[w,v]"S" south central areas and "E" east coast areas.

Table 2. Relation of donor tree condition at time of budwood collection to transmission of tristeza in Mexican lime, exocortis in citron, stem pitting in sour orange, Mme. Vinous sweet orange, Pineapple sweet orange, Duncan grapefruit, *Citrus excelsa*, and Rusk citrange. Summary 1972 and 1973 indexing data.

Transmissible agent and indicator seedling	Rating of donor tree at time of budwood collection			
	0[z]	1/2	1-1 1/2	2-3
Tristeza--Mexican lime	24/24[y](100)[x]	12/12(100)	41/43(95)	23/23(100)
Exocortis--citron	18/23(78)	7/18(40)	28/42(67)	15/20(75)
Stem pitting--sour orange[w]	14/30(47)	5/10(50)	17/34(50)	41/28(50)
Stem pitting--Mme. Vinous	21/33(66)[u]	20/23(87)	30/40(75)	14/23(61)
Stem pitting--Pineapple[v]	9/12(75)	3/3(100)	21/27(78)	13/20(65)
Stem pitting--Duncan grapefruit[v]	8/11(73)	2/2(100)	15/25(60)	6/9(66)
Stem pitting--*Citrus excelsa*[v]	10/11(90)	2/3(66)	22/26(85)	10/12(85)
Stem pitting--Rusk citrange[v]	4/8(50)	4/4(100)	13/23(57)	11/13(85)

[z] 0 = apparently healthy; 1/2 = very early stage of decline; 1-1 1/2 = mid-stage of decline; 2-3 = later stages of decline.

[y] Number of donor trees eliciting the specific response/total donors indexed.

[x] Percentage of "y."

[w] Presumed to be caused by tristeza virus.

[v] From 1973 indexing data.

[u] See "Results" for specific transmission data.

2 donor trees carrying the stem pitting factor have yet to show YTD symptoms. Stem pitting was not transmitted from 12 of the 33 healthy donor trees; of these 12, 6 showed YTD symptoms in 4 to 12 months, and 6 have not yet exhibited symptoms at 1 year after budwood collection. Although Mme. Vinous has been used in Florida for indexing, stem pitting in this cultivar has only recently been reported (6).

Grapefruit. In the 1972 inoculations, stem pitting was transmitted from only 4 of 49 donor trees.

In the 1973 inoculations, stem pitting was transmitted from 31 of 47 donor trees in all groves except those from grove K (Table 1). Donors for the 1973 inoculations were from the same groves indexed in 1972. A slight to moderate chlorosis and/or chlorotic mottle, with slight to moderate stunting, was observed in inoculations from all groves except K. Stem pitting was transmitted from 8 of 11 donor trees rated "0" (Table 2). These 8 trees showed YTD symptoms in 5 to 14 months after budwood collection. No stem pitting was transmitted from the other 3 donors that subsequently showed YTD symptoms in 8 to 14 months. Transmission of stem pitting to grapefruit was generally reduced as YTD-induced stress increased (Table 2).

A slight chlorotic mottle with slight stunting was noted in inoculations with the T_3A tristeza isolate, but neither T_3A nor the T_1M isolate of tristeza produced stem pitting.

Eureka lemon. Sixty-nine trees from 11 groves were indexed. With the exception of an occasional pit in a few seedlings, no stem pitting was observed. Mild to moderate chlorotic mottle and slight stunting, possibly a mild seedling yellows response, was observed in some inoculated seedlings. Psorosis was transmitted from several donors in grove B.

Citrus excelsa. No stem pitting was observed in the 1972 indexing when data were taken 6 months after inoculation. In the 1973 indexing (data taken at 14 months), stem pitting was light to moderate in the newly developed spring wood and was transmitted from 44 of 52 donors from 10 of 11 groves (Table 1). Most of the inoculated seedlings were slightly to moderately stunted and some exhibited a slight chlorotic mottle. Tree condition at time of budwood collection did not appear to have any influence on the transmissibility of the stem pitting agent. The T_1M tristeza isolate caused slight stunting and mild stem pitting in young wood, while the T_3A tristeza isolate caused moderate stunting, chlorotic mottle, and severe stem pitting in young and old wood.

Rusk citrange. Inoculated seedlings showed an occasional non-persistent chlorotic mottle, vein flecking, and slight stunting. Stem pitting, primarily in the spring developed wood, was observed in inoculations from 32 of 48 donors from 11 of

11 groves (Table 1). Stem pitting was not observed in the 1972 indexing. Donors from the 1973 inoculations were from the same groves previously indexed in 1972. Both T_1M and T_3A isolates of tristeza virus produced some stunting, vein flecking, and mild to moderate stem pitting.

Pineapple orange. With the exception of psorosis symptoms in several seedlings (grove B) and some slight nonpersistent chlorotic mottle, most seedlings showed no leaf symptoms. Stem pitting was not observed in the 1972 indexing (49 donors) but mild to moderate stem pitting was observed from 46 of 62 donors in the 1973 indexing (Table 1). This stem pitting was observed in wood developed in late fall or early spring and was primarily confined to terminal portions of the Pineapple seedlings. Donor trees for the 1973 inoculations were from the same groves indexed in 1972.

Transmission of stem pitting was generally reduced as YTD-induced stress increased (Table 2). Stem pitting was transmitted from 9 of 12 donors rated "0" (apparently healthy). These 9 trees showed YTD symptoms in 6 to 13 months after budwood collection. The 3 "healthy" donors that did not produce stem pitting in the Pineapple seedlings exhibited YTD symptoms in 6 to 9 months. Inoculations with T_1M isolate of tristeza virus did not elicit stem pitting, but several small pits were produced with the severe T_3A isolate.

Rough lemon. Sixty-nine trees from 11 groves were indexed. With the exception of an occasional plant with one or several small pits, the inoculated seedlings showed no symptoms.

Dweet tangor. Forty-nine trees from 11 groves were indexed. Stem pitting (one to several small pits) was observed in several seedlings. A slight nonpersistent chlorotic mottle was noted in some of the seedlings and psorosis was observed in some of the inoculations from grove B.

Orlando tangelo. Ninety trees from 11 groves were indexed. No symptoms of xyloporosis have yet been observed.

Discussion

The extensive transmission experiments initiated during 1972 and continued in 1973 and 1974 have not yet established whether YTD is caused by a transmissible factor. Foliage symptoms associated with YTD are not apparent in grove trees until they reach bearing age (5 to 7 years) although bark gumming and pegging (Fig. 1) with corresponding wood pitting can be found in the stock just below the bud union in trees 4 years old. In bearing trees, older than 7 years, bark and wood symptoms in the stock are randomly distributed and are generally present 1 year before foliage symptoms appear. Stress from fruiting, drought, or cold appears to hasten the onset of foliage symptoms.

There are several considerations that should be taken into account in the interpretation of these indexing data: First, in the 1972 inoculations, stem pitting was not observed in the indicator seedlings of grapefruit, *C. excelsa*, Pineapple, and Rusk citrange. These seedlings were inoculated during the summer and data were taken at 6 months for *C. excelsa* and at 10 months (early spring before new growth matured) for the other 3 indicators. We have since observed that stem pitting in these and other indicators is not evident until plants have either gone through winter dormancy and/or there is sufficient new wood development and subsequent maturation at cooler temperatures. One exception to the above has been found in *C. excelsa* when inoculated with the T_3A isolate of the tristeza virus. With this isolate, *C. excelsa* produces severe stem pitting even at summer temperatures of 85 to 95°F. The T_3A isolate, however, is considerably more virulent than the tristeza currently found in the 102 donor trees indexed; second, transmission data from grove K (Red grapefruit), although still incomplete, have not shown stem pitting for *C. excelsa*, sour orange, or grapefruit when compared to the other groves affected by YTD. Trees in this grove, with few exceptions, did not exhibit typical YTD symptoms, particularly the characteristic zinc-like deficiency pattern. We do not have a complete index on the various donor trees in this grove simply because many of our selected donor trees were inadvertently removed. We have since added 3 other grapefruit groves to our program.

Although 8 additional *Citrus* indicator species have been included in the indexing program to find, if possible, other transmissible agents, current indexing data revealed primarily 3 transmissible agents: exocortis, tristeza, and a new Mme. Vinous stem pitting factor. The latter 2 were present in all groves indexed and showed a high incidence of correlation with YTD when budwood for indexing was obtained from donor trees in a very early stage of disease. The inability to transmit the stem pitting agent consistently to Mme. Vinous, Pineapple, and to grapefruit as compared with the transmission of tristeza to Mexican lime from severely declined donor trees would indicate that the stem pitting agent is either a specialized strain of tristeza or a different virus. Whether tristeza or the Mme. Vinous stem pitting aget alone or combined can cause YTD in *Citrus* currently remains to be determined. Dual infections of tristeza with another virus or mycoplasma have been consistently more destructive than tristeza alone (10, 11). Because exocortis virus was transmitted from ca. 70% of the donors, its role, if any, as a potential suspect in the YTD syndrome, currently cannot be overlooked.

We examined trunks, limbs, and small branches of over 400 YTD-affected trees of Valencia, Pineapple, Queen, Hamlin, and grapefruit and found only 9 trees (Valencia, Pineapple, and Queen) with mild stem pitting in several branches of approximately 5 to 10 mm in diameter. Four of these 9 trees had been previously indexed and were found to consistently elicit stem pitting in Mme. Vinous and Pineapple and, inconsistently, in grapefruit seedlings.

Although indexing for xyloporosis is still incomplete, it appears that xyloporosis is not involved as the causal agent, particularly in YTD-affected nucellar Valencia (grove J), because this virus is neither insect- nor seed-transmitted in Florida (3, 4).

At the present time, all but 8 of the initially "healthy" donors selected in the 15 groves included in this study have become diseased within 3 to 24 months after initiating the indexing program. Thus, in spite of the fact that only tentative comparisons of indicator response can be made at this time between transmission data from "healthy" and YTD-affected trees, there currently appears to be evidence for a pattern of indicator responses associated with YTD-affected trees. The validity of these indicator responses, particularly those of Mme. Vinous, Pineapple, grapefruit, and possibly others will depend on the results of current indexing from healthy trees selected from groves unaffected by YTD.

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