Trunk Xylem Anatomy of Mature Healthy and Blighted Grapefruit Trees on Several Rootstocks

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Additional index words. vessel element diameter and density, wood hydraulic conductivity, dye movement, *Citrus paradisi*

Abstract. Wood samples were taken from healthy and blighted citrus trees on several rootstocks to describe and compare the xylem anatomy of the healthy trees and to determine if blight altered xylogenesis. Horizontal trunk xylem cores, 6 cm long, were extracted from blighted 18-year-old commercial grapefruit (*Citrus paradisi* Macf.) trees on rough lemon (RL) (*C. jambhiri* Lush.), Cleopatra mandarin (CM) (*C. resnyi* Hort. ex Tan.), and Carrizo citrange (CC) [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf] and from healthy trees on those rootstocks and sour orange (SO) (*C. aurantium* L.). Cores were taken from the eastern and western sides of the scion and rootstock of each tree. The cores were divided into 2-cm pieces and cross-sections were prepared for analysis of vessel element (VE) number and diameter in 0.5-cm increments. A sample-size study showed that tree side was not a significant source of variation and that 10 replications were sufficient to detect differences of ≈12% from the overall mean. Among the healthy trees, VE densities and diameters were similar for the trees on CC or RL and larger than those for trees on SO or CM. VEs were generally smaller and at lower densities in the scion than the rootstock. Few VE occlusions were observed in the healthy trees. In the blighted trees, to a depth of 1 cm, VE density increased and diameter decreased compared to the healthy trees. The largest change occurred in the trees on RL and in the rootstock vs. scion trunk part. The frequency of VE amorphous plugs in blighted trees ranged from 1% to 30%. Similar changes in xylem anatomy were not found in trees with citrus tristeza virus or soilborne pests. Trunk water uptake and dye movement patterns in blighted trees were typical for trees with xylem dysfunction.

Citrus blight is a wilt disease of unknown etiology that causes trees to decline and become unproductive. This disease occurs in many areas where citrus is grown but has been particularly severe in Florida and Brazil (Beretta et al., 1988). The first visible symptom is reduced shoot growth followed by a general decline of the canopy and eventual fibrous root loss (Albrigo et al., 1986; Wutscher et al., 1982). The decline apparently results from xylem dysfunction. Blighted trees take up virtually no water when tested by injection into the trunk xylem (Albrigo et al., 1986; Brlansky et al., 1984; Cohen, 1979; Young et al., 1978) and conduct little dye when injected into a major root or the trunk (Young and Garnsey, 1977; Young et al., 1979).

Hydraulic conductivity comparisons of healthy and blighted trees in situ and of core samples have consistently shown that some longitudinal water flow occurs in the xylem to a depth of ≈2 to 3 cm into the trunks of mature healthy trees with little or no flow at greater depths and that blighted trees conduct a relatively small amount of water usually only within ≈0.5 cm of the cambium. This difference in the pattern and amount of water flow between healthy and blighted trees remains unexplained but has been associated with vessel element (VE) occlusions (Albrigo et al., 1986; Brlansky et al., 1984). Amorphous plugs occur with greater frequency in the xylem of blighted trees, but their presence has not been proven to be the first or only factor disrupting xylem function.

Xylem function is related to anatomy, particularly VE characteristics. General anatomical features of citrus secondary xylem have been described by de Villiers (1939), Schneider (1968), and Webber and Fawcett (1935), but their reports provided little detailed, comparative information about scion, rootstock, and their reciprocal effects on VEs.

Mendel (1945) linked xylem function and anatomy in a study of ‘Shamouti’ sweet orange transpiration. The size and number of VEs in the wood of trees on Palestine sweet lime (*Citrus limettioides* Tan.) were larger than in trees on sour orange (SO), a result suggesting that this difference may partly explain the greater vigor, drought tolerance, and yield of trees on sweet lime. Trees on other rootstocks such as rough lemon (RL) and Volkamer lemon (*C. volkameriana* Ten. & Pasq.) are also vigorous and highly productive. Rootstocks with those characteristics are among the most susceptible to blight, whereas SO is considered to be tolerant (Castle et al., 1989). Thus, wood anatomy could be related to rootstock tolerance and the general response of citrus trees to blight. Nemec et al. (1975) noted that VE diameter in the young xylem was smaller in blighted trees and suggested that this change from healthy trees was the result of water stress.

Our objectives were to describe and compare trunk xylem anatomy of healthy citrus trees on several rootstocks, to determine if wood anatomy differed in blighted trees on the same rootstocks, and relate any observed anatomical changes to known rootstock responses to blight.

Materials and Methods

Field site and experiment trees. Grapefruit trees in a central Florida commercial grove were selected for study. ‘Marsh’ trees on RL and SO and ‘Redblush’ trees on Carrizo citrange (CC) were located in adjacent blocks of several hundred trees each that were planted in 1972. ‘Duncan’ trees on Cleopatra mandarin (CM) planted in 1978 were in a nearby block. The trees were in north–south rows spaced 4.6 × 7.6 m, except for the ‘Duncan’ trees, which were planted in pairs so that every 4.6 m there were two trees ≈2.4

Received for publication 28 Jan. 1993. Accepted for publication 13 Sept. 1993. Florida Agricultural Experiment Station Journal series no. R-02936. We thank Marvin Kahn and Wayne Douberley of Kahn Grove Service, Sebring, Fla., for providing the site and trees and allowing us considerable flexibility in conducting our experiment. Also, we are indebted to K.S. Derrick, R.H. Brlansky, R.F. Lee, J.W. Noling, L.W. Timmer, L.G. Albrigo, and their assistants for contributions during the study. Special thanks are extended to F.G. Martin for statistical analyses and W.L. Stern for training and advice in the art of microtechnique. Financial support provided by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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m apart instead of one tree. Trunk diameter 15 cm above the bud union was ≈ 25 cm for all trees in the experiment. They received standard commercial care, including irrigation with microsprinklers and annual hedging on both sides along the row. The soil is Astatula fine sand (Typic Quartzipsamment), a deep, well-drained soil commonly planted to citrus.

Xylem core sampling and processing. Each block of trees was mapped and 10 healthy and 10 blighted trees were selected by appearance. There were no blighted trees on SO. Blight diagnosis was confirmed with trunk water uptake by syringe injection (Lee et al., 1984; Lima et al., 1984) and by Zn accumulation in trunk phloem (Albrigo and Young, 1981). Healthy trees on RL and SO were sampled in Dec. 1989. Cores from the other trees were collected in Mar. and Apr. 1990. Radial 6-cm xylem cores were taken from the eastern and western sides of each tree ≈ 7 to 10 cm above and below the bud union with a 5.2-mm-diameter increment borer (Haglof; Forestry Suppliers, Jackson, Miss.). Each core was immediately placed in a vial of formaldehyde–acetic acid–ethanol (FAA) solution and fixed for at least 18 h. The FAA was replaced by 70% ethanol followed by one additional change of alcohol.

Each core was subdivided into 2-cm pieces. Core pieces were observed with a dissecting microscope before sectioning to establish proper orientation. Transverse sections ≈ 30 μ m thick were cut near the center of the core pieces with a sliding microtome (model 860; AO Spencer, Southbridge, Mass.) stained with hematoxylin–safranin, and mounted with balsam on glass slides.

Sample-size estimation. A preliminary study was conducted to determine, from sample-size estimates, the magnitude of the difference in VE density that could be detected with 10 replications and to identify significant sources of variation. RL and SO transections made from a complete set of healthy tree cores were divided into 0.5-cm increments, giving a total of 12 increments per core representing the depths 0 to 0.5 to 5.5 to 6.0 cm. A typical microscope field ($\times 100$) was located within each depth and the number of VEs was counted. An analysis of variance of these data in a split-plot arrangement with rootstock as the main plot factor and trunk part (scion or rootstock), side, and depth as subplot factors revealed that tree side was not a significant factor. Rootstock, trunk part, and depth were highly significant factors; however, the differences among depths were generally $< 5\%$, and the same results were obtained for the three factors when data for depth 0 to 2 cm were compared to data for depths 0 to 3 or 0 to 6 cm. Sample-size estimates ($\alpha = 0.05$, $\beta = 0.10$) indicated that a difference in VE density of $\approx 12\%$ from the general mean could be detected with 10 replications. Thus, tree side was eliminated as a factor and data for the remaining cores were taken from only the 0 to 2 cm depth of 10 replications.

Vessel element data. VE densities were measured as described above for treatments not included in the preliminary study; then, for all treatments, the number of solitary VEs and groupings composed of two to six VEs were counted and the frequency of amorphous plugs was recorded. The tangential and radial lumen diameters of single VEs and each VE occurring in groups of two or three were measured with a microscope (model BH2; Olympus Corp., Cherry Hill, N.J.) coupled to an image analysis system (Bioquant System IV; R&M Biometric, Nashville, Tenn.). The number of grouped or solitary VEs measured was proportional to their frequency. A few typical groups of four to six VEs were also measured. Total VE cross-sectional area (CSA) per square millimeter of xylem was calculated from the density and diameter data. Xylem characteristics were quantified and described according to standard wood anatomy guidelines (Wheeler et al., 1989).

Water uptake and dye movement. Water uptake in 0.5-cm depth

increments was measured in each of the 20 trees on the four rootstocks during Mar. and Apr. 1990. A small square of bark was removed ≈ 20 cm above the bud union, then a 3.2-mm-diameter hole was drilled to a depth of 0.5 cm. A syringe was inserted that projected < 0.2 cm into the hole. After water uptake was measured for 30 sec or less, the hole was enlarged to 0.5 cm and drilled to 1 cm depth. A metal tube (o.d. slightly larger than 0.5 cm) was pushed in 0.5 cm. A syringe was attached to the end of the tube and water was injected. This procedure was repeated to a depth of 6 cm. Immediately afterward, xylem cores were extracted ≈ 3 cm directly below the water injection holes for trees on CC and CM. Because cores had already been taken from trees on RL and SO, water uptake for those trees was measured at locations to the side of the core sampling sites.

In May 1990, dye solution (crystal violet, 0.1% in water, w/v) was injected, as described above, into two healthy and three blighted ‘Marsh’ trees on RL selected from among other trees in the experiment block and into two healthy trees at another location. Each tree was pushed out within several hours and trunk cross-sections were cut at several places above and below the bud union. The upper surface of the section, which included the injection sites, was sanded smooth and examined with a dissecting microscope at $\times 40$. The number of stained and unstained VEs was counted in ≈ 1 -cm² areas 5 cm above each injection site. Examination of the trunk sections showed that the metal tube effectively sealed the wood except for the 0.5-cm depth being injected.

Additional sampling. In Feb. and Mar. 1991, xylem cores were collected and transections prepared as previously described from five sets of trees at sites in other areas of Florida. Four of the sets were 12- to 20-year-old sweet orange trees, in two locations (Indiantown and St. Cloud), on RL, CM, and CC rootstocks and 5-year-old trees on RL. One core was taken from the rootstock of three healthy and three blighted trees diagnosed by water uptake at each site. Also, four each of healthy, blighted, or tristeza-infected (determined previously; R.F. Lee, personal communication) sweet orange trees on SO at an East Coast site (Ft. Pierce) were sampled. The number of VEs was counted and their diameters were measured in all transections.

Soil, root, and leaf samples were collected at the Indiantown site and from each of the original experiment trees to determine the presence of *Phytophthora parasitica* Dast., *Tylenchulus semipenetrans* Cobb (citrus nematode), and citrus tristeza virus (CTV).

Statistical analysis. Data were analyzed with PROC ANOVA (Statistical Analysis System, Cary, N.C.) in a split-plot design with trees as replications. The data satisfied the Huynh–Feldt condition and a repeated measures analysis was not required (Littell, 1989). For the comparison among healthy trees, rootstocks were the main plot factor and trunk part and sampling depth were the subplot factors. Healthy and blighted trees were compared within rootstocks, with tree health as the main plot factor and the same subplot factors as above. Mean separation was by Duncan’s multiple range test where appropriate. The 0.5-cm increment water uptake data for healthy and blighted trees were compared at each depth by Student’s *t* test, $\alpha = 0.05$.

Results and Discussion

General xylem structure of healthy trees

The secondary xylem anatomy of all the transections examined was typical for *Citrus*, as described by Schneider (1968), with regard to fibers, VEs, and parenchyma (Fig. 1 a and b). There were no apparent gross differences in xylem composition or structure.

Growth rings were evident in the wood but they were indistinct and difficult to count. Terminal parenchyma was not distinguishable in our xylem sections.

Crystal idioblasts of calcium oxalate are found in *Citrus* (Goldschmidt-Blumenthal, 1956; Schneider, 1968). We commonly observed crystals, presumably of calcium oxalate, in the parenchyma and ray cells of the xylem examined. They occurred more frequently in the trees on CM, in which crystal size was relatively large as also noted by Nanthachai (1976).

Vessel element characteristics of healthy trees

Density, diameter, and total CSA. Considering all treatments, mean VE density was 18/mm² (Table 1). Solitary VEs had a mean diameter of 80 μ m and those in groups of two or three were smaller. The mean total CSA of VE in 1 mm² of xylem was 77,106 μ m² (Table 1).

Rootstock and trunk part had highly significant effects on each VE variable. Trees on CC and RL had similar VE densities and diameters, which were higher than those for trees on CM and SO (Table 1); also, VE density was always higher in the rootstock. There was a relatively large difference between the rootstock and scion in trees on CC or RL but almost no difference in those on CM or SO. Rootstock effects on VE diameter depended on trunk part; in the rootstock, RL VEs were relatively wide, whereas, in the scion, VEs in the trees on CC had the largest diameter. Depth affected only VE density, which was higher in the younger than the older xylem. The differences with depth in the diameters of VEs occurring in doublets were not the same for each trunk part (Table 1).

Total CSA reflects VE density and diameter. The scion had fewer VEs but their mean diameter was $\approx$ 35% larger than for those in the rootstock; therefore, total CSAs were larger in the scion

Fig. 1. Healthy tree (A) scion and (B) rootstock and blighted tree (C) scion and (D) rootstock transections of trunk xylem, 0 to 0.5 cm deep, from mature 'Marsh' grapefruit trees on rough lemon rootstock. Bar = 100 μ m.

Table 1. Mean (n = 10) vessel element (VE) densities, total cross-sectional areas, and diameters in transverse sections of scion and rootstock xylem taken from the trunks of healthy, mature grapefruit trees on four rootstocks.

Treatment	VE density (no./mm ²)	Total VE cross- sectional area (μm ² ·mm ⁻²)		Diam (μm) of a VE occurring						
				Singly		In a doublet		In a triplet		
Analysis of variance ^z										
Rootstock (R)	0.0001 ^y		0.0001		0.0038		0.0001		0.0002	
Trunk part (P)	0.0001		0.0001		0.0001		0.0001		0.0001	
R × P	0.0002		0.0001		0.0008		0.0001		0.0001	
Depth (D)	0.0584		NS		NS		NS		NS	
R × D	NS		NS		NS		NS		NS	
P × D	NS		NS		NS		0.0035 ^x		NS	
R × P × D	NS		NS		NS		NS		NS	
Grand mean	18.4		77,106 ^w		80		72		67	
CV (%)	21		25		21		23		27	
Main effect means										
R										
Rough lemon (RL)	19.4		88,283		84		76		70	
Sour orange (SO)	16.3		67,478		79		71		65	
Cleopatra mandarin (CM)	15.6		59,119		76		67		63	
Carrizo citrange (CC)	22.4		93,541		79		73		68	
P										
Scion (S)	16.2		88,504		92		82		74	
R	20.6		65,706		68		62		59	
D (cm)										
0 to 0.5	19.4		79,516		79		70		66	
0.5 to 1.0	18.2		78,792		81		73		68	
1.0 to 1.5	18.6		77,383		79		72		67	
1.5 to 2.0	17.5		72,730		80		71		67	
Interaction means										
R × P	S	R	S	R	S	R	S	R	S	R
RL	15.7 b ^v	23.2 a	82,860 b	93,705 a	90 ab	78 a	81 b	71 a	70 b	69 a
SO	15.3 b	17.3 b	79,801 b	55,155 c	89 b	69 b	80 b	62 b	73 b	58 b
CM	14.1 b	17.1 b	76,311 b	41,927 d	91 ab	61 c	81 b	54 c	73 b	53 b
CC	19.6 a	25.1 a	115,043 a	72,039 b	96 a	63 c	86 a	60 b	80 a	57 b

^zSplit-plot design.

^yProbabilities of significant F values where nonsignificant (NS) = $P > 0.1$.

^xSee text for explanation.

^wMean values/10,000 = percentage VE area in 1 mm² of xylem.

^vMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

except for RL (Table 1). The combined effects of VE density and diameter resulted in a significantly larger total CSA in the rootstock vs. the scion of trees on RL. The proportion of VE to xylem CSA ranged from ≈4% (CM rootstock part) to >11% (CC scion part) with an overall mean of 7.7%.

The general results from wood anatomy studies in South Africa (de Villiers, 1939), Israel (Goldschmidt-Blumenthal, 1956), and Florida (Nanthachai, 1976) support our observation that VE density is greater in the rootstock than the scion and that the reverse is true for VE diameter; however, the VE densities reported in the earlier studies were usually higher and VE sizes were smaller than those measured in our study. Variations in plant material age and environment could account for these differences.

Grouping size and distribution. VEs occurred singly and in radial clusters of two to six elements. Solitary VEs plus those in groups of two (doublet) or three (triplet) accounted for 90% to 97% of the total number of VEs. Solitary VEs were ≈20% to 26% of the total and doublets and triplets were ≈45% to 52% and 15% to 30%, respectively. Trees on SO and CM tended to have higher proportions of solitary VEs and fewer triplets.

Grouping size affected VE diameters based on pooled data from

healthy and blighted trees (Table 2). The mean diameter of solitary VEs was ≈15% more than for VEs in a triplet; however, there was considerable but similar variation within each grouping. The smallest VEs were 35 to 40 μm in diameter and the largest ones were ≈120 μm.

Occlusions. No amorphous plugs were observed in the VEs of trees on RL, and those on SO or CC had <0.3% VEs plugged. The proportion of plugging was significantly larger (1.2%) in trees on CM (Tables 3–5).

Vessel element characteristics of blighted trees

Rough lemon. There were more VEs and VEs were smaller in blighted than healthy trees; however, the major differences in VE density and diameter occurred in the 0 to 0.5 cm depth and in the rootstock (Table 3). As a result, there were many highly significant interactions among the treatments.

VE density in blighted trees varied >4-fold over sample depth in the rootstock. The small increase in VE density between depths 2 to 1 cm suggested a change was occurring over time, but the largest difference was between 0 and 1 cm. VE density also increased in the scion from depths 2 to 0 cm but the change was less

Table 2. Variation in the diameter of vessel elements (VE) in scion (S) and rootstock (R) xylem taken from the trunks of mature grapefruit trees on four rootstocks.

Rootstock	Trunk part	Diam ² (μm) for a VE occurring								
		Singly			In a doublet			In a triplet		
		Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Rough lemon	S	87	10	62–119	77	8	48–110	75	10	47–100
	R	76	11	43–106	71	10	39–98	68	10	39–96
Sour orange	S	89	10	64–117	80	9	60–103	73	10	48–97
	R	70	9	49–98	62	7	41–84	58	9	33–84
Cleopatra mandarin	S	91	10	60–116	80	8	62–100	75	10	57–118
	R	61	9	38–89	54	8	36–72	52	8	35–72
Carrizo citrange	S	94	11	66–123	85	9	67–106	78	11	49–108
	R	63	8	44–90	59	7	44–86	56	8	41–81

²Means, sds, and ranges are based on data pooled for 10 healthy and 10 blighted trees and four observations, made at 5-mm increments, along the xylem trunk cores (sample depth); thus, n = 80.

Table 3. Mean (n = 10) vessel element (VE) densities, total cross-sectional areas, percentage plugging, and diameters in transverse sections of scion and rootstock xylem taken from the trunks of healthy and blighted mature grapefruit trees on rough lemon rootstock.

	VE density		Amorphous		Total VE cross- sectional		Diam (μm) of a VE occurring					
Treatment	(no./mm ²)		plugs (%)		area (μm ² /mm ⁻²)		Singly		In a doublet		In a triplet	
Analysis of variance ^z												
Tree health (H)	0.0001 ^y		--- ^x		0.0001		0.077		NS		NS	
Trunk part (P)	0.0001		---		0.0039		0.0001		0.0001		0.0007	
H × P	0.0001		---		NS		NS		0.0408		NS	
Depth (D)	0.0001		---		0.0001		0.0008		0.0121		0.0001	
H × D	0.0001		---		0.0001		0.0001		0.0001		0.0684	
P × D	0.0001		---		0.0924		0.1002		0.0004		0.0136	
H × P × D	0.0001		---		0.0015		NS		0.0398		NS	
Grand mean	25.9		3.8		104,966 ^w		82		74		72	
CV (%)	39		---		25		20		17		21	
Main effect means												
H												
Healthy (H)	19.4		0.0		88,283		83		75		70	
Blighted (B)	32.4		7.6		121,647		81		74		73	
P												
Scion (S)	19.7		4.7		96,248		87		78		75	
Rootstock (R)	32.1		2.9		113,681		76		71		69	
D (cm)												
0 to 0.5	41.8		1.9		133,228		77		70		67	
0.5 to 1.0	22.1		4.9		102,693		84		77		75	
1.0 to 1.5	20.7		4.4		97,371		84		76		74	
1.5 to 2.0	19.0		3.9		86,568		83		77		73	
Interaction means												
H × P × D	S	R	S	R	S	R	S	R	S	R	S	R
H												
1	17.6	24.3	0.0	0.0	93,540	93,540	92	78	83	72	74	67
2	15.2	21.3	0.0	0.0	82,362	93,540	92	79	84	74	75	73
3	15.3	23.5	0.0	0.0	80,009	96,481	88	77	77	70	76	67
4	14.6	23.6	0.0	0.0	74,714	91,186	87	78	78	72	72	68
B												
1	32.9	92.6	6.6	1.0	137,074	208,258	81	56	72	55	74	54
2	24.5	27.5	11.6	9.0	109,424	124,720	85	82	76	76	77	76
3	21.3	22.6	9.4	8.1	107,071	105,306	89	84	78	79	78	75
4	16.2	21.4	10.4	5.1	84,715	95,305	91	78	83	76	80	73

²Split-plot design.

^yProbabilities of significant F values where nonsignificant (NS) = $P > 0.1$.

^xData were not analyzed because of the zero values for healthy trees.

^wMean values/10,000 = percentage VE area in 1 mm² of xylem.

Table 4. Mean (n = 10) vessel element (VE) densities, total cross-sectional areas, percentage plugging, and diameters in transverse sections of scion and rootstock xylem taken from the trunks of healthy and blighted mature grapefruit trees on Cleopatra mandarin rootstock.

	VE density		Amorphous		Total VE cross-sectional		Diam (μm) of a VE occurring ^z		
Treatment	(no./mm ²)		plugs (%)		area (μm ² ·mm ⁻²)		Singly	In a doublet	In a triplet
Analysis of variance ^y									
Tree health (H)	NS ^x		0.0001		0.0898		NS	NS	NS
Trunk part (P)	0.0039		NS		0.0001		0.0001	0.0001	0.0001
H × P	NS		NS		NS		NS	NS	0.0296
Depth (D)	NS		0.0029		NS		0.0732	0.0047	0.0137
H × D	0.0011		0.0046		0.0029		NS	NS	NS
P × D	NS		NS		NS		NS	NS	NS
H × P × D	NS		NS		NS		NS	NS	NS
Grand mean	16.2		12.5		62,107 ^w		76	67	64
CV (%)	23		83		28		24	23	33
Main effect means									
H									
Healthy (H)	15.6		1.2		59,119		76	67	63
Blighted (B)	16.7		23.8		65,095		76	68	65
P									
Scion (S)	15.1		12.0		81,741		91	81	76
Rootstock (R)	17.3		13.0		42,472		61	54	53
D (cm)									
0 to 0.5 cm	16.9		8.1		61,226		74	64 b ^y	62 b ^y
0.5 to 1.0	16.0		12.2		63,793		77	69 a	67 a
1.0 to 1.5	15.7		16.2		57,837		75	66 b	62 b
1.5 to 2.0	16.0		13.4		65,571		79	71 a	66 a
Interaction means									
H × P × D	S	R	S	R	S	R			
H									
1	13.7	16.0	0.4	1.0	70,596	35,298			
2	13.4	16.3	0.0	1.8	74,714	45,299			
3	15.9	18.2	1.0	0.7	82,362	42,946			
4	13.6	17.8	4.4	0.0	77,656	44,122			
B									
1	18.1	19.9	13.3	17.7	90,010	49,417			
2	15.6	18.8	23.6	23.2	90,598	44,123			
3	14.1	14.8	32.2	30.9	74,126	31,768			
4	16.1	16.6	20.8	28.5	94,128	47,064			

^zMeans are not given where an interaction was not significant.

^ySplit-plot design.

^xProbabilities of significant F values where nonsignificant (NS) = $P > 0.1$.

^wMean values/10,000 = percentage VE area in 1 mm² of xylem.

^vMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

abrupt and smaller in magnitude than in the rootstock.

The proportion of VE to xylem CSA increased nearly 50% from 8.8% to 12.1% in blighted compared to healthy trees. The changes in VE density were greater than the decreases in diameter; thus, the total CSA of blighted trees was almost always larger than that of the healthy trees.

The mean frequency of amorphous plugs in blighted trees was 7.6%, and plugs were found at all depths in the scion and rootstock samples, with the lowest percentage at the 0 to 0.5 cm depth (Table 3).

Cleopatra mandarin. Small but similar increases in VE density occurred between 2 and 1 cm in the blighted scion and rootstock trunk parts, whereas there were no changes in the healthy trees (Table 4). The younger xylem tended to have smaller VE diameters, but the differences with depth were not consistent or affected by blight. Mean total CSA was larger in the scion because of larger

VE diameters in the scion, but the difference between healthy and blighted trees was not significant.

The blighted trees had a significantly higher frequency of plugging than healthy trees; also, the plugs were about equally distributed in the scion and rootstock of blighted trees, except that fewer were found in the youngest xylem.

Carrizo citrange. VE density was higher in blighted than healthy trees and in the rootstock than the scion with a gradual increase between 2 and 0 cm (Table 5); however, the part × depth interaction was significant, indicating a greater change over depth in the rootstock than the scion. Tree health had no effect on VE diameter, except that the solitary elements in the scion of the blighted trees decreased from a diameter of 102 μm in the older xylem to 84 μm in the 0 to 0.5 cm deep xylem. Total VE CSA of the blighted trees was larger than that of the healthy trees, but the difference was significant at a statistical probability slightly larger

Table 5. Mean (n = 10) vessel element (VE) densities, total cross-sectional areas, percent plugging, and diameters in transverse sections of scion and rootstock xylem taken from the trunks of healthy and blighted mature grapefruit trees on Carrizo citrange rootstock.

	VE density		Amorphous		Total VE cross-sectional		Diam (μm) of a VE occurring ^z		
Treatment	(no./mm ²)		plugs (%)		area (μm ² ·mm ⁻²)		Singly In a doublet In a triplet		
Analysis of variance ^y									
Tree health (H)	0.0504 ^x		0.0001		0.0714		NS		NS
Trunk part (P)	0.0038		NS		0.0001		0.0001		0.0001
H × P	NS		NS		NS		NS		NS
Depth (D)	0.0015		0.044		0.0268		NS		0.0024
H × D	NS		0.0649		NS		NS		NS
P × D	0.0759		NS		NS		0.0208		NS
H × P × D	NS		NS		NS		0.0172		NS
Grand mean	23.7		7.8		96,913 ^w		79		72
CV (%)	21		97		25		22		23
Main effects means									
H									
Healthy (H)	22.4		0.3		93,541		79		73
Blighted (B)	25.0		15.3		100,285		79		72
P									
Scion (S)	21.2		7.6		120,964		95		85
Rootstock (R)	26.2		8.0		72,862		63		59
D (cm)									
0 to 0.5	26.4		4.6		101,756 a ^v		76		68 b
0.5 to 1.0	25.0		8.7		102,902 a		79		73 a
1.0 to 1.5	22.7		8.6		95,168 ab		80		74 a
1.5 to 2.0	20.6		9.3		87,824 b		80		74 a
Interaction means									
H × P × D	S	R	S	R	S R				
H									
1	20.8	26.0	0.0	0.2	97 ^{NS}		62 ^{NS}		
2	20.2	26.6	1.3	0.0	94		63		
3	18.5	27.0	0.3	0.2	95		67		
4	19.1	20.8	0.0	0.5	97		62		
B									
1	28.5	30.1	10.2	8.2	84 b		63 ^{NS}		
2	22.9	30.3	17.2	16.1	93 ab		67		
3	19.6	25.7	16.0	18.0	97 ab		63		
4	19.9	22.8	16.1	20.5	102 a		60		

^zMeans are not given where an interaction was not significant.

^ySplit-plot design.

^xProbabilities of significant F values where nonsignificant (NS) = $P > 0.1$.

^wMean values/10,000 = percentage VE area in 1 mm² of xylem.

^vMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

than the 5% level usually accepted.

There were more amorphous plugs in blighted than healthy trees and their distribution was affected by depth (Table 5). The percentage of plugging ranged from 8% to 20.5% in the blighted trees, with the lowest values occurring in the 0 to 0.5 cm depth of the scion and rootstock parts.

Additional xylem cores; leaf and soil samples

The differences in VE density and diameter observed between the blighted and healthy trees on RL, CM, and CC were also observed among the additional rootstock xylem samples collected from trees on the same rootstocks elsewhere in Florida (data not given). VE density for the trees on SO, in the 0 to 0.5 cm depth, was 37.5, 19.8, and 19.4/mm² for the blighted, healthy, and CTV-infected trees, respectively. VE diameters were smaller in the blighted than healthy trees.

Phytophthora parasitica and *T. semipenetrans* were not detected in any of the soil or root samples, except there were high levels of *Phytophthora* in the experiment trees on CM. The trees at Indiantown were infected with a mild strain of CTV but none was detected in the experiment trees.

Water uptake and dye movement

Water uptake. The xylem water uptake patterns in 0.5-cm depth increments were similar among the healthy trees on the four rootstocks (Fig. 2). Uptake was highest in the youngest xylem (0 to 1 cm) and decreased to 0 at ≈3.5 cm into the trunk. Trees on RL and CC took up more water in the 0 to 1 cm depth than those on SO or CM. There was no water uptake in the blighted trees except for a small amount in the 0 to 0.5 cm depth that was significantly less than that for the healthy trees.

Dye movement. The pattern (Fig. 3) and quantities of dye

Fig. 2. Mean ($n = 10$) water uptake in 0.5-cm depth increments in the scion trunk part of healthy (●) and blighted (○) mature grapefruit trees on several rootstocks. There were no blighted trees on sour orange rootstock. Bars represent $\pm$ SE and, where present, indicate significant differences between the healthy and blighted trees within a rootstock and depth based on Student's t test.

absorbed were essentially the same as those for water. Dye was rapidly taken up in the first 0.5 cm of xylem in the healthy trees and moved ≈ 50 cm up and 25 cm down the trunk within 1 to 2 min after injection. Little dye was taken up at depths > 2 cm and none was taken up at 6 cm. Where dye was absorbed, it spread tangentially. This was particularly evident at the 0- to 3-cm injection site. The number of stained VEs at each depth also paralleled the water uptake pattern. Virtually all VEs were stained at the 0- to 1-cm depth, less than one-half of the VEs stained at the intermediate depths, and none stained at 6 cm.

Relationship of xylem anatomy and rootstock to blight

Our principal findings were that the pattern of xylogenesis was altered in blighted citrus trees and that VE characteristics were different among healthy grapefruit trees on four rootstocks.

The changes in VE number and diameter are a new blight symptom. They are clearly not the cause of blight, only a manifestation; nevertheless, their occurrence introduces new possibilities regarding blight etiology. One such possibility concerns the citrus tree hormonal system, which was first implicated when Bausher (1982) reported that the levels of indoleacetic acid oxidase were lower in blighted than in healthy citrus trees. Further evidence of a blight-hormone connection has been obtained from mineral

Fig. 3. Trunk section from a healthy 18-year-old 'Marsh' grapefruit tree syringe injected 10 cm above the bud union with 0.1% crystal violet dye. The section was collected ≈ 2 h after injections at depths of (in cm) (A) 0 to 3, (B) 0 to 0.5, (C) 0.5 to 1.0, (D) 1.0 to 1.5, (E) 1.5 to 2.0, (F) 2.5 to 3.0, (G) 4.5 to 5.0, and (H) 5.5 to 6.0. The surface of the section is ≈ 5 cm above the injection plane.

element redistribution studies, which have shown that Zn accumulates in trunk xylem and phloem (Williams and Albrigo, 1984). Zinc is involved in auxin metabolism, and accumulation sites in blighted trees may likewise be sites of altered auxin activity.

Our results are consistent with a blight effect on hormone physiology and considerably strengthen this connection. Xylem tissue is the end product of cambial activity and cytodifferentiation (Savidge, 1985). Both of these components of xylogenesis are considered to be under hormonal, primarily auxin, control (Aloni and Zimmermann, 1983; Roberts et al., 1988; Savidge, 1990a, 1990b). Differences in VE size and density similar to those we measured in healthy trees have been induced experimentally with exogenous auxin applications (Pizzolato, 1981; Roberts et al., 1988).

The changes in the pattern of xylogenesis were sufficiently consistent to be a diagnostic symptom and one that precedes the appearance of visual symptoms. Wood anatomy in all of the blighted trees studied was affected to a depth of 1 cm, which is ≈ 2 years' growth, as estimated from trunk measurements made in a nearby rootstock experiment. Blighted trees at the field site were routinely removed each year based on visible symptoms; therefore, the xylem changes were probably detectable at about the same time as Zn begins to accumulate and protein-related changes occur but before xylem dysfunction (Albrigo et al., 1986; Derrick et al., 1990; Wutscher et al., 1982).

Apart from the diagnostic value of the changes in VE density and diameter, the relative magnitude of those changes as they occurred in the rootstock may be the more important aspect. Among the rootstocks included in our study, the most blight-susceptible rootstock (RL) had the greatest change in the

pattern of xylogenesis compared to the blight-tolerant rootstocks (SO and CM). Therefore, further study with additional rootstocks may establish if their VE characteristics, based on changes that occur between healthy and blighted trees on a given rootstock, have a predictable relationship to blight tolerance.

One of the original diagnostic symptoms of citrus blight was reduced water uptake. Therefore, because water flow in trees is partially a function of xylem anatomy, the VE differences among the rootstocks studied are useful in explaining the behavior among healthy and blighted trees.

Our healthy trees differed primarily in VE size and number. Diameter is the one dimension most affecting flow volume and conductive efficiency (Zimmermann, 1983). Wide VEs are more efficient than narrow ones (Carlquist, 1975). Trees on RL and CC had larger and more VEs and higher water uptake rates and are more vigorous and productive than those on SO, which had smaller and fewer VEs (Castle et al., 1989). Trees on CM are often as vigorous as those on RL but do not fruit as heavily as young trees (Castle et al., 1989). The smaller VEs and CSAs of the former may not be capable of supplying enough water to young trees to support growth and fruiting until some conductive threshold is reached as the trees age. Field leaf ψ (Castle and Krezdorn, 1977; Crocker et al., 1974) and seedling root hydraulic conductivity data (Syvertsen and Graham, 1985) support these comparisons among rootstocks.

Water flow in the blighted trees occurred only in the youngest 0.5 cm of xylem and at significantly lower rates than in the healthy trees. This effect of blight on water flow at the 0 to 0.5 cm depth seems to contradict expected increases in conductivity as a result of the larger VE total CSA reported herein for the blighted trees. However, because small changes in VE size have large effects on water flow (Zimmermann, 1983), the net effect of reduced VE diameter in the blighted trees despite increased VE number and total CSA may have been a loss in conductivity.

Impairment of xylem function in blighted citrus trees is a consistent observation considered to be the result of vessel plugging (Cohen et al., 1983); however, the exact involvement of plugs in xylem dysfunction and the factors influencing their formation are unknown. Occlusions occur throughout the wood of blighted trees and in the youngest tissue but only occasionally in the wood of healthy trees. Correlations of water uptake with plug counts have yielded coefficients that range from about -0.4 to -0.6 (Albrigo et al., 1986; Brlansky et al., 1984; Timmer et al., 1986, 1988). The extent of unexplained variation suggests that other factors are involved in loss of xylem function (Wutscher, 1989).

Loss of xylem function in blighted citrus trees and in the older wood of healthy trees may be caused by cavitation and the formation of emboli, events that along with water flow are related to VE characteristics. Cavitation occurs commonly in forest trees and other plants as a result of water stress and winter freezing (Tyree and Sperry, 1989) but has not been considered in citrus blight. Cavitation, if occurring naturally in citrus trees, might explain why the older wood in healthy trees does not apparently conduct water, yet few plugs are found there (Timmer et al., 1986). In blighted trees, cavitation and embolism may precede VE plugging, a sequence observed in diseased elm trees (Newbanks et al., 1983). Loss of vessel water has caused tyloses to form (Zimmermann, 1983), and emboli formation may be likewise related to plugging in citrus trees.

Emboli formation has a known relationship to VE characteristics (Tyree and Sperry, 1989). Wide VEs conduct water more efficiently than narrow ones, but the latter are less susceptible to cavitation. Citrus trees with narrow VEs may be protected in some way from xylem dysfunction. Such an association exists between

VEs and rootstock blight tolerance. Trees on those rootstocks with narrow VEs are either tolerant to blight (SO) or are affected as older trees (CM); those with wider VEs (RL and CC) are susceptible.

Our results define a new symptom with possible value in predicting rootstock blight tolerance. Healthy trees of a susceptible rootstock, like RL, had a higher VE density and larger VEs than those on tolerant rootstocks such as CM and SO; and, the susceptible rootstock had a relatively greater change in its pattern of xylogenesis as a result of blight. The new symptom broadens the spectrum of known physical plant responses and provides clues about the physiological changes in blighted trees.

Literature Cited

- Albrigo, L.G. and R.H. Young. 1981. Phloem zinc accumulation in citrus trees affected with blight. *HortScience* 16:158-160.
- Albrigo, L.G., J.P. Syvertsen, and R.H. Young. 1986. Stress symptoms of citrus trees in successive stages of decline due to blight. *J. Amer. Soc. Hort. Sci.* 111:465-470.
- Aloni, R. and M.H. Zimmermann. 1983. The control of vessel size and density along the plant axis: A new hypothesis. *Differentiation* 24:203-208.
- Bausher, M.G. 1982. Changes in IAA-oxidase activity and the severity of citrus blight. *HortScience* 17:497. (Abstr.)
- Beretta, M.J.G., A.F.V. LeFevre, and W.B.C. Moraes. 1988. Xylem plugging and water flow in citrus seedlings and grafted plants in relation to decline. *Proc. 10th Intl. Organization Citrus Virol. Conf.* p. 377-380.
- Brlansky, R.H., L.W. Timmer, R.F. Lee, and J.H. Graham. 1984. Relationship of xylem plugging to reduced water uptake and symptom development in citrus trees with blight and blightlike declines. *Phytopathology* 74:1325-1328.
- Carlquist, S.J. 1975. *Ecological strategies of xylem evolution*. Univ. of California Press, Berkeley.
- Castle, W.S. and A.H. Krezdorn. 1977. Soil water use and apparent root efficiencies of citrus trees on four rootstocks. *J. Amer. Soc. Hort. Sci.* 102:403-406.
- Castle, W.S., D.P.H. Tucker, A.H. Krezdorn, and C.O. Youtsey. 1989. Rootstocks for Florida citrus. *Florida Coop. Ext. Serv. Bul.* SP-42.
- Cohen, M. 1979. Hydraulic conductivity of wood of trees with citrus blight. *Proc. Florida State Hort. Soc.* 92:68-70.
- Cohen, M., R.R. Pelosi, and R.H. Brlansky. 1983. Nature and location of xylem blockage structures in trees with citrus blight. *Phytopathology* 73:1125-1130.
- Crocker, T.E., W.D. Bell, and J.F. Bartholic. 1974. Scholander pressure bomb technique to assess the relative leaf water stress of 'Orlando' tangelo scion as influenced by various citrus rootstocks. *HortScience* 9:453-455.
- Derrick, K.S., R.F. Lee, R.H. Brlansky, L.W. Timmer, B.G. Hewitt, and G.A. Barthe. 1990. Proteins associated with citrus blight. *Plant Dis.* 74(2):168-170.
- Goldschmidt-Blumenthal, S. 1956. Rootstock-scion influences in the morphology and anatomy of the budunion of Shamouti orange. *Bul. Res. Council Israel* 5D:143-154.
- Lee, R.F., L.J. Marais, L.W. Timmer, and J.H. Graham. 1984. Syringe injection of water into the trunk: A rapid diagnostic test for citrus blight. *Plant Dis.* 68:511-513.
- Lima, J.E.O., L.A.B.C. Vasconcellos, and J. Pompeu, Jr. 1984. Use of a simple device to test the specificity of the water-injection test for decline of citrus. 1984 *Proc. Intl. Soc. Citricult.* 1:402-404.
- Littell, R.C. 1989. Statistical analysis of experiments with repeated measures. *HortScience* 24:37-40.
- Mendel, K. 1945. Orange leaf transpiration under orchard conditions. Part 2: Soil moisture content decreasing. *Palestine J. Bot. Rehovot Ser.* 5:59-85.
- Nanthachai, P. 1976. Comparative studies of rootstock-scion combinations of citrus with special reference to anatomical structure. MS thesis. Univ. of Florida, Gainesville.
- Nemec, S., R. Constant, and M. Patterson. 1975. Distribution of obstruc-

- tions to water movement in citrus with and without blight. *Proc. Florida State Hort. Soc.* 88:70–75.
- Newbanks, D., A. Bosch, and M.H. Zimmermann. 1983. Evidence for xylem dysfunction by embolization in Dutch elm disease. *Phytopathology* 73:1060–1063.
- Pizzolato, T.D. 1981. Anatomical modification by 2,4-DB of vascular cambium and secondary xylem in soybean internodes. *Can. J. Bot.* 60:2142–2146.
- Roberts, L.W., P.B. Gahan, and R. Aloni. 1988. Vascular differentiation and plant growth regulators. Springer-Verlag, New York.
- Savidge, R.A. 1985. Prospects for manipulating vascular-cambium productivity and xylem-cell differentiation, p. 208–227. In: M.G.R. Cannell and J.E. Jacksons (eds.). *Attributes of trees as crop plants*. I.T.E. Abbots Ripton, England.
- Savidge, R.A. 1990a. Characterization of indol-3-ylactic acid in developing secondary xylem of 26 Canadian species by combined gas chromatography-mass spectrometry. *Can. J. Bot.* 68:521–523.
- Savidge, R.A. 1990b. Phytohormone regulation of secondary xylem development. *Proc. Intl. Union For. Res. Organization XIX World Congr. Div. S*:65–76.
- Schneider, H. 1968. The anatomy of citrus, p. 1–85. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). *The citrus industry*. vol. 2. Univ. California Press, Berkeley.
- Syvertsen, J.P. and J.H. Graham. 1985. Hydraulic conductivity of roots, mineral nutrition, and leaf gas exchange of citrus rootstocks. *J. Amer. Soc. Hort. Sci.* 110:865–869.
- Timmer, L.W., R.H. Brlansky, J.H. Graham, H.A. Sandler, and J.P. Agostini. 1986. Comparison of water flow and xylem plugging in declining and in apparently healthy citrus trees in Florida and Argentina. *Phytopathology* 76:707–711.
- Timmer, L.W., R.H. Brlansky, J.H. Graham, J.P. Agostini, and R.F. Lee. 1988. Effect of amorphous plugs, filamentous plugs and depth into trunk xylem on the water flow in declining and healthy citrus trees. *Proc. 10th Intl. Organization Citrus Virol. Conf.* p. 370–376.
- Tyree, M.T. and J.S. Sperry. 1989. Vulnerability of xylem to cavitation and embolism. *Annu. Rev. Plant Physiol.* 40:19–38.
- de Villiers, E. 1939. The xylem anatomy, relative water conductivity and transport of dyes in citrus stems. *S. African J. Sci.* 36:291–313.
- Webber, I.E. and H.S. Fawcett. 1935. Comparative histology of healthy and psorosis-affected tissues of *Citrus sinensis*. *Hilgardia* 9(2):71–109.
- Wheeler, E.A., P. Baas, and P.E. Gasson. 1989. IAWA list of microscopic features for hardwood identification. *Intl. Assn. Wood Anatomy Bul. New Ser.* 10(3):219–332.
- Williams, G.L. and L.G. Albrigo. 1984. Some inorganic element changes in trunk phloem of healthy and blight-affected citrus trees. *J. Amer. Soc. Hort. Sci.* 109:437–440.
- Wutscher, H.K. 1989. Long-term patterns of water uptake in syringe injections and wood zinc levels of blight-affected orange trees. *Proc. Florida State Hort. Soc.* 102:24–27.
- Wutscher, H.K., P.E. Smith, and F. Bistline. 1982. Zinc accumulation in the trunk wood and the development of visual symptoms of citrus blight. *HortScience* 17:676–677.
- Young, R.H. and S.M. Garnsey. 1977. Water uptake patterns in blighted citrus trees. *J. Amer. Soc. Hort. Sci.* 102:751–756.
- Young, R.H., S.M. Garnsey, and G. Horanic. 1979. A device for infusing liquids into the outer xylem vessels of citrus trees. *Plant Dis. Rpt.* 63(9):713–715.
- Young, R.H., H.K. Wutscher, M. Cohen, and S.M. Garnsey. 1978. Citrus blight diagnosis in several scion variety/rootstock combinations of different ages. *Proc. Florida State Hort. Soc.* 91:56–59.
- Zimmermann, M.H. 1983. Xylem structure and the ascent of sap. Springer-Verlag, New York.