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

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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. reshni 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., 1982; Nemec et al., 1975; Young et al., 1979). The diffusion of 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 Volkmann 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

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Sample-size estimates (α = 0.05, β = 0.10) indicated that a power of 0.80 for the comparison among healthy trees, rootstocks were the main plot factor and trunk part (scion or rootstock), side, and depth as subplot in a split-plot arrangement with rootstock as the main plot factor and data for the remaining cores were taken from only the 0 to 0.5 cm depth being injected. Thus, tree side was eliminated as a factor and 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 the 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 ×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., Tylanchulus 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, α = 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 µm and those in groups of two or three were smaller. The mean total CSA of VE in 1 mm² of xylem was 77,106 µm² (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 ≈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 = 10 µm.
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 $\approx 4\%$ (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 $\mu m$ in diameter and the largest ones were $=120\mu m$.

**Oclusions.** 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 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./mm²) | Total VE cross-sectional area (µm²·mm⁻²) | Diam (µm) of a VE occurring | Singly | In a doublet | In a triplet |
|-----------|---------------------|-------------------------------------------|-----------------------------|--------|--------------|-------------|
|           |                     |                                           |                             |        |              |              |
| Analysis of variance† |                     |                                           |                             |        |              |              |
| Rootstock (R)    | 0.0001*             | 0.0001                                   | 0.0038                      | 0.0001 | 0.0001       | 0.0001       |
| Trunk part (P)   | 0.0001              | 0.0001                                   | 0.0001                      | 0.0001 | 0.0001       | 0.0001       |
| R × P            | 0.0002              | 0.0001                                   | 0.0008                      | 0.0001 | 0.0001       | 0.0001       |
| Depth (D)        | 0.0584              | NS                                        | NS                          | NS     | NS           | NS           |
| R × D            | NS                  | NS                                        | NS                          | NS     | NS           | NS           |
| P × D            | NS                  | NS                                        | NS                          | NS     | 0.0035†      | NS           |
| R × P × D        | NS                  | NS                                        | NS                          | NS     | NS           | NS           |
| Grand mean       | 18.4                | 77,106*                                   | 80                          | 72     | 67           |
| CV (%)           | 21                  | 25                                        | 21                          | 23     | 27           |

Main effect means

| R           | Rough lemon (RL) 19.4 | 88,283 | 84 | 76 | 70 |
| Sou (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 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 |

*Split-plot design.
†Probabilities of significant F values where nonsignificant (NS) = $P > 0.1$.
‡See text for explanation.
§Mean values/10,000 = percentage VE area in 1 mm² of xylem.
\*Mean separation within columns by Duncan’s multiple range test at $P ≤ 0.05$. 

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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 | Singly | Diam³ (µm) for a VE occurring | 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    |

³Means, sd's, 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.

| Treatment                      | VEs density (no./mm²) | Amorphous cross sectional plugs (%) | Total VE cross-sectional area (µm²/mm²) | Diam (µm) of a VE occurring |
|--------------------------------|-----------------------|-------------------------------------|----------------------------------------|-----------------------------|
|                                |                       |                                     |                                        | Singly | In a doublet | In a triplet |
| Analysis of variance           |                       |                                     |                                        | 0.0001  | 0.0001      | 0.0007       |
| Tree health (H)                |                       |                                     |                                        | 0.0007  | NS           | NS           |
| Trunk part (P)                 |                       |                                     |                                        | NS      | 0.0408      | NS           |
| H × P                          |                       |                                     |                                        | NS      | 0.0001      | NS           |
| Depth (D)                      |                       |                                     |                                        | 0.0008  | 0.0121      | 0.0001       |
| H × D                          |                       |                                     |                                        | 0.0001  | 0.0001      | 0.0684       |
| P × D                          |                       |                                     |                                        | 0.1002  | 0.0004      | 0.0136       |
| Grand mean                     |                       |                                     |                                        | 25.9    | 3.8         | 104.966w     |
| CV (%                          |                       |                                     |                                        | 39      | --          | 25           |
| Main effect means              |                       |                                     |                                        |         |             |              |
| H Healthy (H)                  |                       |                                     |                                        | 19.4    | 0.0         | 88,283       |
| Blighted (B)                   |                       |                                     |                                        | 32.4    | 7.6         | 121,647      |
| P Scion (S)                    |                       |                                     |                                        | 19.7    | 4.7         | 96,248       |
| Rootstock (R)                  |                       |                                     |                                        | 32.1    | 2.9         | 113,681      |
| D (cm)                         |                       |                                     |                                        |         |             |              |
| 0 to 0.5                       |                       |                                     |                                        | 41.8    | 1.9         | 133,228      |
| 0.5 to 1.0                     |                       |                                     |                                        | 22.1    | 4.9         | 102,693      |
| 1.0 to 1.5                     |                       |                                     |                                        | 20.7    | 4.4         | 97,371       |
| 1.5 to 2.0                     |                       |                                     |                                        | 19.0    | 3.9         | 86,568       |
| Interaction means              |                       |                                     |                                        |         |             |              |
| H × P × D                      |                       |                                     |                                        |         |             |              |
| H 1                            |                       |                                     |                                        | 17.6    | 24.3        | 0.0          |
|                                |                       |                                     |                                        | 93,540  | 93,540      | 92           |
|                                |                       |                                     |                                        | 78      | 83          | 74           |
|                                |                       |                                     |                                        | 72      | 74          | 67           |
| 2                              |                       |                                     |                                        | 15.2    | 21.3        | 0.0          |
|                                |                       |                                     |                                        | 82,362  | 93,540      | 92           |
|                                |                       |                                     |                                        | 79      | 84          | 74           |
|                                |                       |                                     |                                        | 75      | 73          |              |
| 3                              |                       |                                     |                                        | 15.3    | 23.5        | 0.0          |
|                                |                       |                                     |                                        | 80,009  | 96,481      | 88           |
|                                |                       |                                     |                                        | 77      | 77          | 76           |
|                                |                       |                                     |                                        | 70      | 76          | 67           |
| 4                              |                       |                                     |                                        | 14.6    | 23.6        | 0.0          |
|                                |                       |                                     |                                        | 74,714  | 91,186      | 87           |
|                                |                       |                                     |                                        | 78      | 78          | 72           |
|                                |                       |                                     |                                        | 72      | 72          | 68           |
| B 1                            |                       |                                     |                                        | 32.9    | 92.6        | 6.6          |
|                                |                       |                                     |                                        | 137,074 | 208,258     | 81           |
|                                |                       |                                     |                                        | 56      | 72          | 74           |
|                                |                       |                                     |                                        | 54      |              |              |
| 2                              |                       |                                     |                                        | 24.5    | 27.5        | 11.6         |
|                                |                       |                                     |                                        | 109,424 | 124,720     | 85           |
|                                |                       |                                     |                                        | 82      | 76          | 76           |
|                                |                       |                                     |                                        | 77      |              |              |
| 3                              |                       |                                     |                                        | 21.3    | 22.6        | 9.4          |
|                                |                       |                                     |                                        | 107,071 | 105,306     | 89           |
|                                |                       |                                     |                                        | 84      | 78          | 78           |
|                                |                       |                                     |                                        | 79      |              |              |
| 4                              |                       |                                     |                                        | 16.2    | 21.4        | 10.4         |
|                                |                       |                                     |                                        | 84,715  | 95,305      | 91           |
|                                |                       |                                     |                                        | 78      | 83          | 76           |
|                                |                       |                                     |                                        | 76      |              | 80           |
|                                |                       |                                     |                                        | 73      |              |              |

³Split-plot design.
³³Probabilities of significant F values where nonsignificant (NS) = P > 0.1.
³³³Data were not analyzed because of the zero values for healthy trees.
³³⁴Mean 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.

| Treatment            | VE density (no./mm$^2$) | Amorphous plugs (%) | Total VE cross-sectional area ($\mu$m$^2$·mm$^{-2}$) | Diam (μm) of a VE occurring$^a$ |
|----------------------|-------------------------|----------------------|-----------------------------------------------------|---------------------------------|
|                      |                         |                      |                                                    | Singly | In a doublet | In a triplet |
|                      |                         |                      |                                                    |        |              |              |
| Analysis of variance$^b$ |                         |                      |                                                    |        |              |              |
| Tree health (H)      | NS$^c$                  | 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$^v$ | 62 b$^v$
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
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

$^a$Means are not given where an interaction was not significant.
$^b$Split-plot design.
$^c$Probabilities of significant F values where nonsignificant (NS) = $P > 0.1$.
$^w$Mean values/10,000 = percentage VE area in 1 mm$^2$ of xylem.

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...
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

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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.

| Treatment | VE density (no./mm²) | Amorphous plugs (%) | Total VE cross-sectional area (µm²·mm⁻²) | Diam (µm) of a VE occurring* |
|-----------|-------------------|---------------------|------------------------------------------|-----------------------------|
|           |                   |                     |                                          | Singly | In a doublet | In a triplet |
| Analysis of variance<sup>y</sup> |                   |                     |                                          |       |             |             |
| Tree health (H) | 0.0504<sup>x</sup> | 0.0001              | 0.0714                                  | NS    | NS          | NS          |
| Trunk part (P)  | 0.0038            | NS                  | 0.0001                                  | 0.0001| 0.0001      | 0.0001      |
| H × P          | NS                | NS                  | NS                                       | NS    | NS          | NS          |
| Depth (D)      | 0.0015            | 0.044               | 0.0268                                  | NS    | NS          | NS          |
| H × D          | NS                | 0.0649              | NS                                       | NS    | NS          | NS          |
| P × D          | 0.0759            | NS                  | NS                                       | 0.0208| NS          | NS          |
| H × P × D      | NS                | NS                  | NS                                       | 0.0172| NS          | NS          |
| Grand mean     | 23.7              | 7.8                 | 96,913*                                 | 79    | 72          | 67          |
| CV (%)         | 21                | 97                  | 22                                       | 22    | 23          | 26          |

Main effects means

| H | Healthy (H) | 22.4 | 0.3 | 93,541 | 79 | 73 | 68 |
|   | Blighted (B) | 25.0 | 15.3| 100,285| 79 | 72 | 67 |
| P | Scion (S)    | 21.2 | 7.6 | 120,964| 95 | 85 | 79 |
|   | Rootstock (R)| 26.2 | 8.0 | 72,862 | 63 | 59 | 56 |
| D (cm) | 0 to 0.5 | 26.4 | 4.6 | 101,756 | 76 | 68 | b 68 |
|     | 0.5 to 1.0  | 25.0 | 8.7 | 102,902 | 79 | 73 | a 67 |
|     | 1.0 to 1.5  | 22.7 | 8.6 | 95,168 | 80 | 74 | a 67 |
|     | 1.5 to 2.0  | 20.6 | 9.3 | 87,824 | 80 | 74 | a 68 |

Interaction means

| H × P × D | S | R |
|-----------|---|---|
| H         |   |   |
| 1         | 20.8 | 26.0 | 0.0 | 0.2 | 97<sup>NS</sup> | 62<sup>NS</sup> |
| 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<sup>NS</sup> |
| 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 |

<sup>x</sup>Means are not given where an interaction was not significant.
<sup>y</sup>Split-plot design.
<sup>x</sup>Probabilities of significant F values where nonsignificant (NS) = P > 0.1.
<sup>z</sup>Mean values/10,000 = percentage VE area in 1 mm² of xylem.
<sup>o</sup>Mean separation within columns by Duncan’s multiple range test at P ≤ 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.
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 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

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**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 ±SE and, where present, indicate significant differences between the healthy and blighted trees within a rootstock and depth based on Student’s t test.

**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.
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 $\psi$ (Castle and Krezdorn, 1977; Crocker et al., 1974) and seedling root hydraulic conductivity data (Svyrtsen 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; Bransky 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.

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