Comparison of Water Flow and Xylem Plugging in Declining and in Apparently Healthy Citrus Trees in Florida and Argentina

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ABSTRACT

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Citrus blight (CB) in Florida and citrus *declinamiento* (CD) in Argentina are declines of unknown etiology brought about by xylem dysfunction. Water flow and plugging were determined to a depth of 4 cm from the cambium in cores taken from trunks of trees. In healthy trees, water flow was high and amorphous plugs were rare in all segments. In CB- and CD-affected trees, there was little or no water flow in the core segments deeper than 1 cm from the cambium, and amorphous plugs were numerous. In Argentina, apparently healthy but low-vigor (LV) trees on rough lemon, Rangpur lime, Cleopatra mandarin, and sweet orange rootstocks took up little water by the syringe injection method, water conductivity was normal only in the segment 0–1 cm from the cambium, and amorphous plugs were common. When examined by scanning electron microscopy, plugs in LV trees were typical of those in CB and CD trees. Thus, even these LV trees in Argentina may be affected by the disease. Affected trees in Argentina on the highly susceptible trifoliate orange rootstock decline, but those on less susceptible rootstocks apparently produce enough unplugged vessels each year in this humid, subtropical area to avoid sufficient plugging of the xylem to cause canopy decline. There was an inverse relationship between rootstock susceptibility and water flow through the outer 2 cm of trunk wood in Argentina.

Citrus blight (CB) has caused extensive losses in Florida citrus orchards, especially during the last 20 yr. Disease losses have been greatest in orchards on rough lemon (*Citrus jambhiri* Lush) rootstock, where losses may vary from 1 to 20% per year after the orchard is 10 yr old (11,14). Trees on Rangpur lime (*C. limonia* Osb.) and trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and some of its hybrids are also susceptible, but those on Cleopatra mandarin (*C. reticulata* Blanco), sour orange (*C. aurantium* L.), and sweet orange (*C. sinensis* (L.) Osb.) rootstocks tolerate the disease well (14).

The cause of CB is unknown, but it has been transmitted experimentally (13), indicating the disease is infectious. Although the decline symptoms are not diagnostic, many characteristics of the disease are well established. Zinc accumulates in the phloem and outer xylem of the trunks of CB-affected trees (3,10,15). Water uptake into the trunk by gravity infusion (6) or syringe injection (9) is severely reduced compared with healthy trees. Filamentous and amorphous plugs occur in the xylem of CB-affected trees (4), but the restriction of water flow appears to be primarily associated with the presence of amorphous plugs (5). Amorphous plugs are common in CB-affected trees and have not been associated with other citrus tree declines (4,8).

Citrus *declinamiento* (CD, also known as *fruta bolita*) is similar to CB in many respects. Zinc accumulates in trunk wood, water uptake into the trunk is restricted, and amorphous plugs are common in the xylem of CD-affected trees (5,12,16). However, there are some differences between the two diseases. CD may affect trees as young as 3 yr old and has rapidly eliminated all orchards on trifoliate orange rootstock in the province of Misiones, Argentina (1,12). The pattern of rootstock susceptibility is similar to that of CB in Florida, except CD has not seriously affected trees in commercial orchards on rough lemon and Rangpur lime rootstocks in Argentina. Trees on these rootstocks have not declined but are not as vigorous and productive as might be expected, and water uptake into the trunk by gravity flow or injection is low (12; H. K. Wutscher, USDA, Orlando, FL,

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unpublished). These apparently healthy trees with reduced water uptake are subsequently referred to as low-vigor (LV) trees.

The purpose of this study was to compare the water flow and obstructions in the xylem vessels of CB-affected, CD-affected, healthy, and LV trees on several rootstocks to ascertain the basis for the apparent tolerance of rough lemon and Rangpur lime rootstocks to CD in Argentina.

MATERIALS AND METHODS

Samples from Florida were collected from CB-affected and healthy trees from a 15- to 20-yr-old orchard of Hamlin sweet orange on trifoliate orange rootstock near Ft. Pierce, FL, and from a 25- to 30-yr-old orchard of Valencia sweet orange on rough lemon rootstock near Auburndale, FL. Samples of CD-affected Valencia sweet orange on trifoliate orange rootstock and LV trees of the same cultivar on Cleopatra mandarin, sweet orange, rough lemon, and Rangpur lime rootstocks were collected in a rootstock trial near Eldorado, Misiones, Argentina, planted in 1970. Because none of the trees in the rootstock trial on trifoliate orange appeared healthy, samples from a few remaining healthy Valencia sweet orange on trifoliate orange rootstock were obtained from a nearby orchard of about the same age. The number of trees sampled in each instance is listed in Table 1. All samples were collected from the main trunk 15–30 cm above the bud union.

Wood samples for zinc analysis were collected, dried, and analyzed by atomic absorption spectroscopy as described by Wutscher et al (15).

The syringe injection method (9) was used to measure water uptake into the trunk. Water was injected for 30 sec into a small hole drilled into the trunk of the tree, and data were expressed in milliliters taken up per second.

Horizontal core samples about 5 cm long were taken from the scion trunk wood with a 5-mm-diameter Haglof increment borer (Forestry Suppliers, Jackson, MS) directly above the hole drilled for the syringe water injection test. Cores were fixed overnight in 3% glutaraldehyde in 0.066 M sodium-potassium phosphate buffer, pH 6.8. The glutaraldehyde solution was removed, and the cores were stored in the phosphate buffer at 4 C.

Water flow through cores fixed as above was determined in four 1-cm segments measured from the cambium with a device described and presented diagrammatically elsewhere (2). The apparatus consisted of metal tubing 4.25 mm in diameter with the ends of the tubes cut so that they could be clamped snugly around the curvature of the top and the bottom of the core. The 4.25-mm-diameter opening was centered over the desired core segment, the core was oriented in the direction of normal water flow, and a vacuum of 917 mbar was applied. Water was drawn through each core segment for 30 sec, and the flow in milliliters per second calculated.

After water flow determinations, transverse sections 30–50 μm thick were cut from the center one-third of each segment with an AO Spencer Model 860 sliding microtome. The filamentous and amorphous plugs in 200 vessels were counted in random microscope fields at 100×. Sections were prepared for scanning electron microscopy as described previously (4).

TABLE 1. Zinc content of trunk wood and water uptake into tree trunks of healthy (H), declining (D), and low-vigor (LV) citrus trees in Florida and Argentina

Location Scion rootstock[v]	Tree condition	No. of trees	Zn[w] (μg/g)	Trunk water uptake[x] (ml/sec)
Florida				
Val/RL	H	6	1.7	0.68
	D	8	16.0*[y]	0.01*
Florida				
Ham/trif	H	6	4.8	0.81
	D	6	9.4*	0.00*
Argentina				
Val/trif	H	5	1.7	0.98
	D	8	4.1*	0.00*
Argentina				
Val/sweet	LV	5	8.9	0.22 a[z]
Val/Cleo	LV	6	···	0.07 bc
Val/Rang	LV	6	···	0.11 b
Val/RL	LV	5	10.7	0.05 c

[v] Val = Valencia sweet orange, RL = rough lemon, Ham = Hamlin sweet orange, trif = trifoliate orange, sweet = Comun sweet orange, Cleo = Cleopatra mandarin, and Rang = Rangpur lime.

[w] Zn content of trunk wood; one test per tree using duplicate 1-g samples; ··· = no data collected.

[x] Water uptake into trunk as measured by the syringe injection technique (9); one test per tree.

[y] * = Significantly different from the corresponding healthy control trees according to Student's *t* test ($P \leq 0.05$).

[z] Mean separation by Duncan's multiple range test ($P \leq 0.05$).

RESULTS

Water flow was high in all segments of healthy trees on trifoliate orange and rough lemon rootstocks in Florida and of the healthy trees on trifoliate orange rootstock in Misiones, Argentina (Fig. 1A,D,G), but flow declined with distance from the cambium. There were very few or no amorphous plugs in these trees (Fig. 1B,E,H). In the declining trees in Florida and Argentina, water flow was significantly lower than in the corresponding segments in healthy trees (Fig. 1). Water flow was low or nil in all segments except in the segment at 0–1 cm in declining trees from Florida. In nearly all instances, the number of amorphous plugs was significantly greater in declining than in corresponding core segments from healthy trees. There was a significant difference in the number of filamentous plugs between healthy and declining trees in all four segments in the Valencia trees on trifoliate orange rootstock in Argentina (Fig. 1I). In the trees from Florida, the difference was significant in some of the segments of the Hamlin trees on trifoliate orange rootstock (Fig. 1C) and the Valencia trees on rough lemon rootstock (Fig. 1F). Zinc levels were significantly higher in declining than in healthy trees in all cases (Table 1).

The LV trees on Cleopatra mandarin, sweet orange, rough lemon, and Rangpur lime rootstocks in the rootstock trial in Eldorado, Misiones, differed from the healthy and the declining trees described above. Syringe water uptake was greater than that in the declining trees but much less than that in the above healthy trees (Table 1) and lower than the threshold of 0.3 ml/sec established for healthy trees (9). Water flow through core segments of trees on these four rootstocks (Fig. 1J) was comparable to that in healthy trees in the segment at 0–1 cm (Fig. 1A,D,G). Water flow in the core segment at 1–2 cm for the LV trees on Cleopatra mandarin or sweet orange rootstocks was comparable to that in healthy trees in Florida and Argentina, but flow was low for trees on Rangpur lime or rough lemon rootstocks. There was little water flow through the core segments at 2–3 and 3–4 cm taken from trees on any of these four rootstocks (Fig. 1J). The number of amorphous plugs was variable, but amorphous plugs were fewer in the segment at 0–1 cm in the trees on sweet orange and Cleopatra mandarin than on trees on Rangpur lime and rough lemon rootstocks (Fig. 1K).

Water flow in the segment at 0–1 cm of LV trees on rough lemon in Argentina was 0.22 ml/sec (Fig. 1J) or about the same as that of healthy trees on rough lemon in Florida and nearly twice that of the declining trees in Florida (0.12 ml/sec) (Fig. 1D).

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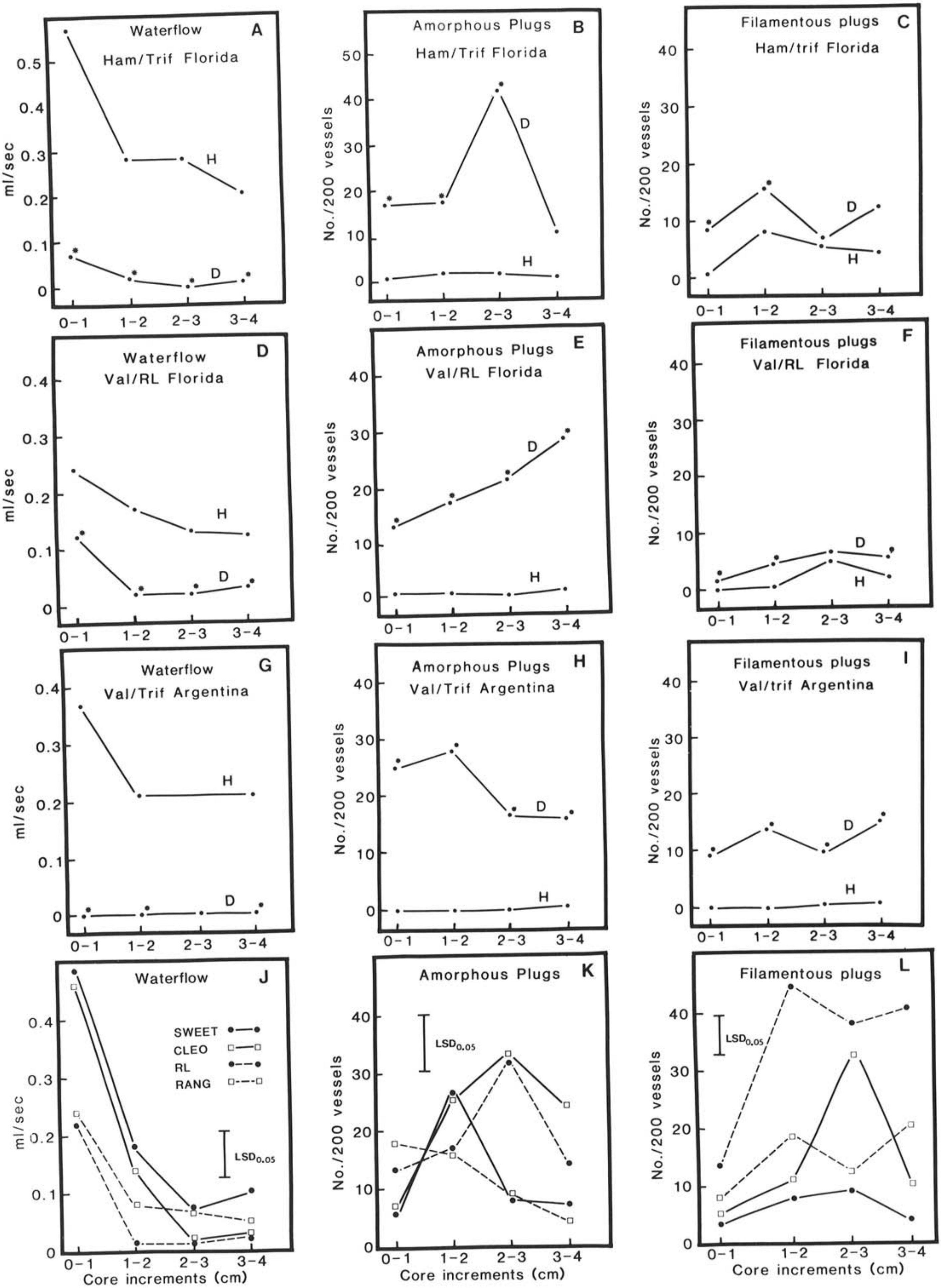
Fig. 1. Water flow and numbers of amorphous and filamentous plugs in core segments of xylem taken 0–1, 1–2, 2–3, and 3–4 cm from the cambium of Valencia (Val) or Hamlin (Ham) sweet orange on rough lemon (RL), trifoliate orange (Trif), Cleopatra mandarin (Cleo), Rangpur lime (Rang), or sweet orange (sweet), rootstocks. **A–F,** Plugs from healthy (H) and citrus blight-affected (D) trees in Florida; **G–I,** plugs from healthy (H) and citrus *declinamiento*-affected (D) trees in Argentina; and **J–L,** plugs from healthy appearing but low-vigor trees in Argentina. * = Mean significantly different from the corresponding healthy trees according to Student's *t* test, $P \leq 0.05$.


Waterflow
Ham/Trif Florida
A
ml/sec
Amorphous Plugs
Ham/Trif Florida
B
No./200 vessels
Filamentous plugs
Ham/trif Florida
C
Waterflow
Val/RL Florida
D
Amorphous Plugs
Val/RL Florida
E
Filamentous plugs
Val/RL Florida
F
Waterflow
Val/Trif Argentina
G
Amorphous Plugs
Val/Trif Argentina
H
Filamentous plugs
Val/trif Argentina
I
Waterflow
J
SWEET
CLEO
RL
RANG
LSD0.05
Amorphous Plugs
K
Filamentous plugs
L
Core increments (cm)
0-1
1-2
2-3
3-4

Linear correlation coefficients for water uptake by the syringe injection method and water flow in the segments at 0–1, 1–2, 2–3, and 3–4 cm were 0.48, 0.65, 0.84, and 0.68, respectively. When water flow in each segment was related to the log of the number of plugs, there were significant correlations ($P \leq 0.05$) of −0.49, −0.44, and −0.48 for amorphous, filamentous, and total plugs, respectively.

Amorphous plugs in the segment at 0–1 cm on LV trees were morphologically identical to the amorphous plugs from the interior of a CB tree from Florida. LV trees in Argentina on rough lemon and sweet orange rootstocks had amorphous plugs (Fig. 2A,D) identical to those found in CB trees in Florida (Fig. 2B) and those in CD trees in Argentina (Fig. 2C).

DISCUSSION

These results support those of our previous studies (5) that amorphous rather than filamentous plugs are associated with restricted water flow. Filamentous plugs were common in declining trees but were not numerous in all diseased trees (Fig. 1C,F,I,L). As in previous work (5), the number of amorphous plugs was negatively correlated with water flow. The number of filamentous plugs was not as well correlated with water flow as was the number of amorphous plugs. Adding the number of filamentous plugs to the number of amorphous plugs did not improve the correlation coefficient. Water uptake measured by the syringe injection technique was better correlated to water flow in

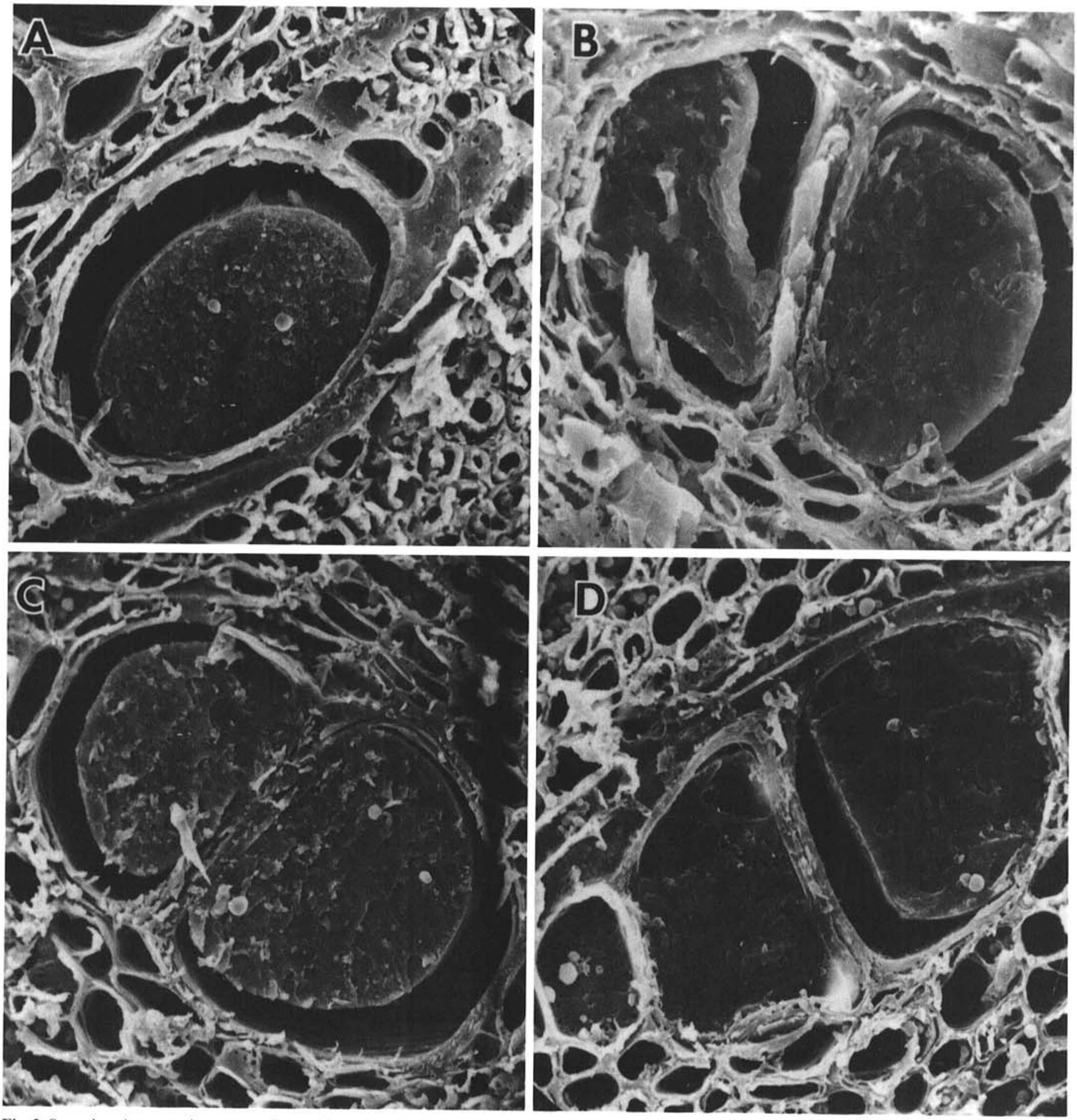


Fig. 2. Scanning electron microscopy of amorphous plugs in the xylem of the core segment 0–1 cm from the cambium of Valencia sweet orange. **A,** Plug from a low-vigor (LV) tree on rough lemon rootstock in Argentina; **B,** plug from a citrus blight-affected tree on rough lemon rootstock in Florida; **C,** plug from a citrus *declinamiento*-affected tree on trifoliate orange rootstock in Argentina; and **D,** plug from an LV tree on sweet orange rootstock in Argentina. All 600×.

the segment at 2–3 cm than in the segments at 0–1, 1–2, or 3–4 cm. In earlier work (5), we had presumed that water uptake by syringe injection measured water flow at a depth of about 2–3 cm. In this study, water uptake was most highly correlated with water flow in this increment and was significantly but less well correlated with water flow in the other increments.

In a previous study on a limited number of trees, using other methods to measure water flow, Cohen et al (7) found that water flow in CB-affected trees occurred only in the outer centimeter of xylem and that very few plugs were located in that area. We found more amorphous plugs and only limited water flow in the outer centimeter, but otherwise, our findings concur with those of the previous study. The amorphous plugs found in the outer centimeter were identical to those found at greater depths.

Water uptake in LV trees is not as severely impeded as that in CB and CD trees but is greatly reduced compared with that in healthy trees. Canopy symptoms in LV trees are similar to those in very mildly affected CB and CD trees, but the decline does not progress to the moderate and severe stages as in CB and CD. The amorphous plugs found in LV trees on Rangpur lime, rough lemon, Cleopatra mandarin, and sweet orange rootstocks are morphologically identical to those in CD- and CB-affected trees in Argentina and Florida. Zinc levels are also high, although we have no truly healthy trees with which to compare them. Because LV trees have reduced water uptake and true amorphous plugs in xylem vessels, characteristics that do not occur in other citrus tree declines (4,8,9), it is our opinion that these trees are affected by CD. The reason that trees on the above four rootstocks do not show severe decline remains uncertain. We found no indication that the lack of decline was due to the types or nature of the plugs in xylem vessels. The only difference we could find was that water flow in the outer centimeter of wood in LV trees on rough lemon rootstock in Argentina was twice that of the declining CB trees on the same rootstock in Florida. We have also found that water uptake in LV trees is greater in summer (February) than in late winter (September), when trees are growing less actively (J. P. Agostini, *unpublished*). As we suggested previously (12), new xylem may form rapidly enough in this warm, humid area to maintain some water flow and to prevent decline.

Under conditions in Misiones, Argentina, even trees on Cleopatra mandarin and sweet orange rootstocks, which are generally considered tolerant or resistant, develop amorphous plugs and restricted water flow. However, water flow in trees on these rootstocks is not reduced as much as in trees on the more susceptible rough lemon or Rangpur lime rootstocks. Indeed, water flow in the outer 2 cm of trees seems to be related to the susceptibility of rootstocks to the disease. Evaluation of germ plasm for resistance to these declines might best be carried out under these conditions.

At present, growers in Misiones have abandoned trifoliate orange as a rootstock. Hybrids of trifoliate orange also appear to be affected by CD at an early age (1). Trees on rough lemon and Rangpur lime rootstocks are reasonably productive, but because these trees are heavily plugged and have low internal water flow, we cannot eliminate the possibility that they may succumb to decline after stresses such as prolonged cold or drought that would suppress formation of new xylem. Cleopatra mandarin and sweet orange appear to be more likely prospects as rootstocks for long-lived orchards in Misiones.

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