Nature and Location of Xylem Blockage Structures in Trees with Citrus Blight

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ABSTRACT

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The relation of hydraulic conductivity to incidence of plugging in xylem vessels was studied in citrus trees with blight. Wood disks were cut from trunks, scaffold limbs, and large roots of cultivar Valencia orange trees on three rootstocks. Pegs, punched out along the diameter of the disks, beginning 0.5 cm inside the cambium and thereafter at 2-cm intervals, were used for conductivity measurements and for determination of numbers of vessel plugs present. In healthy trees conductivity was high and incidence of plugging low except near the pith. Trees with blight disease had high conductivity and little plugging near the cambium, but there was low hydraulic conductivity and much plugging in older xylem tissue. Amorphous plugs were dominant across most of the cross section of blighted trees and appeared to be the characteristic blockage structure found in trees with blight. Filamentous plugs were found mainly near the pith. Conductivity of twigs and pencil-sized roots from healthy and diseased trees was very similar, but vessel plugs were seen almost exclusively in twigs and roots from trees with citrus blight.

Additional key words: young tree decline.

Trees with citrus blight are characterized by greatly reduced uptake of water injected into their trunks (4) and by reduced hydraulic conductivity of wood 1 cm or more inside the cambium (5,12,13). Two types of plugging structures in lumens of xylem vessels from trees with citrus blight may be responsible for the decrease of water uptake.

The first type of occlusion consists of a mass of fine fibers varying from 0.2 to 0.7 μm in diameter (1,2,6,9). Plugs of this kind obstruct the vessel lumens partially or completely and are usually found at the perforation rims, which are located where vessel members join. These plugs have variously been described as hyphae of *Physoderma* (3), lipid plugs (7), and filamentous plugs (10). In this paper the term filamentous plugs (Fig. 1) is used for these fibrous occlusions.

The second type of plug found in vessels in citrus wood consists of solid material that may completely block the vessel lumen or merely line the vessel wall. This blockage structure may be found at any location within the vessel and has been called either gum (8) or an amorphous plug (10). The term amorphous plug will be used here (Fig. 1). Filamentous and amorphous plugs were also found in the vessel lumens of healthy-appearing trees (8,10).

The incidence of amorphous plugs in the wood of trees with citrus blight is higher than in healthy trees (8). Roots of trees with blight have a higher percentage of plugging than healthy trees and an exceptionally high proportion of filamentous plugs occurs near the pith of both healthy and blighted trees (8). Increase in vessel plugging in roots 3–12 mm in diameter from both blighted and healthy trees are associated with a reduction in hydraulic conductivity (8).

The present study began as an investigation of the hydraulic conductivity of the xylem of various parts of Valencia orange trees, with and without blight, on three different rootstocks (5). The purpose of the work reported here was to compare histologically the two types of occlusions found within the xylem vessels of healthy and diseased trees and to determine how these occlusions are related to hydraulic conductivity.

MATERIALS AND METHODS

Cultivar Valencia sweet orange trees (*Citrus sinensis* (L.) Osbeck), 15 yr old, on three rootstocks, Cleopatra mandarin (*C. reticulata* Blanco), sweet orange, and rough lemon (*C. jambhiri* Lush) were selected for study. One healthy tree and two trees with citrus blight on each rootstock were used. Disease status was confirmed by water injection (4) and zinc content (11) diagnostic tests. Disks were cut from trunks, scaffold limbs, and large roots and processed to obtain wood pegs along the disk diameter as previously described (5). Pegs were punched out at 2-cm intervals along the diameter of disks except that the outermost pegs were 0.5

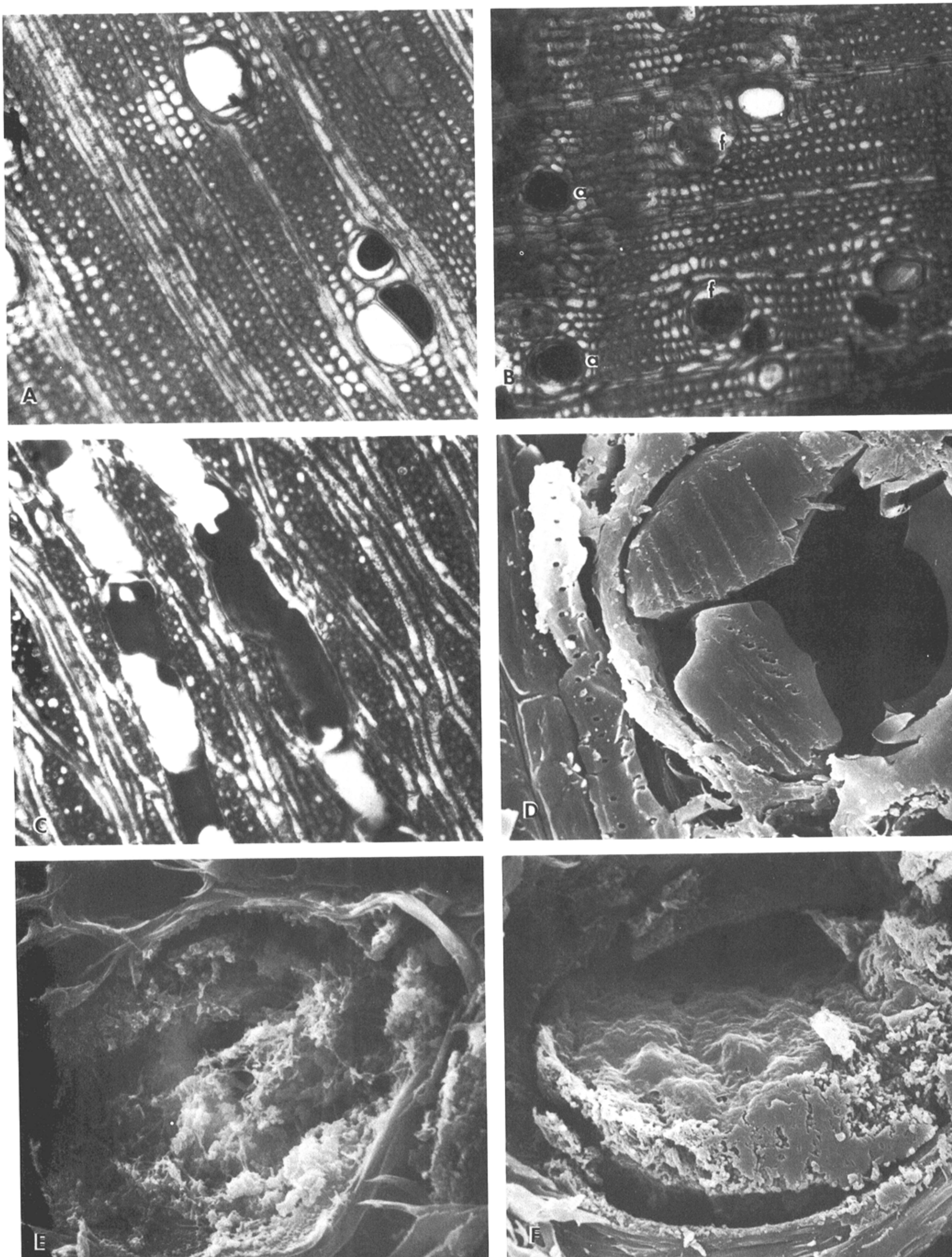

Fig. 1. Vessel plugging in trunks of Valencia orange trees with citrus blight. **A–C,** Light microscopy showing: **A,** amorphous xylem plugging 6 cm from cambium (cross section, ×100); **B,** filamentous (f) and amorphous (a) plugs near trunk center (cross section, ×100); **C,** amorphous plugs 0.5 cm from cambium (longitudinal-tangential section, ×100); **D–F,** Scanning electron microscopy (all cross sections) showing: **D,** amorphous plug, fractured, 8 cm from cambium, ×1,000); **E,** filamentous plug, near trunk center (×1,300); **F,** a plug seeming to combine features of both the amorphous and filamentous types 8 cm from cambium (cross sections, ×2,000).

cm from the cambium and the central peg included the pith. All pegs were 0.8 cm in diameter and 1.25 cm long. Five twigs and five pencil-sized roots (0.6–1.2 cm in wood diameter and 1.25 cm long) also were collected from each tree.

Hydraulic conductivity of pegs, twigs, and small root segments was first determined (5), then all wood pieces used were placed in FAA fixative (a mixture of formalin, glacial acetic acid, ethyl alcohol, and distilled water (1:1:3:5, v/v). Cross sections and longitudinal sections, 25 μm thick, were cut on a Spencer sliding microtome and stained with a 2.5% hematoxylin solution in 95% ethyl alcohol. Cross sections were examined at ×100. The numbers of amorphous and filamentous plugs were determined in the vessels of five randomly located microscope fields, each 2 mm in diameter. All plugs were counted whether they partially or completely blocked the vessel lumen. In most cases, peg sections taken from only half the diameter of the wood disk were examined. Longitudinal sections were used for reference.

Sections from two 1.25-cm segments from each twig and small root sample were examined for incidence of xylem vessel plugs. When vessel plugs in small roots were counted, fields were selected among the larger vessels of the outer half of the root radius to avoid the frequent high concentration of small plugged vessels near the pith.

To prepare tissue for scanning electron microscopy (SEM) sections were washed for 20 min in 0.06 M phosphate buffer, pH 6.8, prior to dehydration. Sections were dehydrated in a 35–100% acetone series for 20 min each and then transferred to an acetone:Freon (2:1 and 1:2, v/v) series for 20 min each. Finally the specimens were transferred into two changes of 100% Freon and critical-point dried on a Bomar critical-point drier (The Bomar Co., Tacoma, WA 98401). The specimens were mounted on SEM stubs, sputter coated with 10^3 nm (100 Å) gold-palladium, and viewed in a JEOL-JSM 35 scanning electron microscope. Six specimens were viewed for each sample, and the most prevalent type of occlusion was noted and photographed.

RESULTS

Amorphous plugs were stained blue-black with the 2.5% hematoxylin in 95% alcohol. In cross section with light microscopy, the amorphous plug material was uniformly dark and without texture (Fig. 1A). In longitudinal section, amorphous plugs varied in length and could be found anywhere within the vessel (Fig. 1C). With the SEM, amorphous plugs appeared to be solid blocks (Fig. 1D). Filamentous plugs with the light microscope appeared granular or fibrous in cross sections (Fig. 1B). In longitudinal view filamentous plugs were largely confined to the perforation plates, were lens-shaped, and did not occlude the central portion of the vessel (1,2,6). The hematoxylin stain usually darkened the outer layers of the filamentous plug but the central portion near the perforation plate remained unstained, giving the plug a sandwich-like appearance. SEM and electron microscope photos of filamentous plugs showed their fibrous nature (Fig. 1E). Most of the vessel plugs examined with light microscopy appeared to be either amorphous or filamentous, but a few plugs, seen with the SEM, appeared to combine amorphous and filamentous characteristics (Fig. 1F). The significance of these structures is not known.

Healthy trees on all three rootstocks usually had few or no plugs in the vessels of scaffold limbs, trunks, and large roots except at centers (Fig. 2). Filamentous plugs were almost exclusively near the pith at the center of the wood disks.

The distribution of vessel plugs in wood disks from trees with citrus blight was very different from that in healthy trees. Trees with blight on all three rootstocks had a high incidence of amorphous plugs in the main body of the disk, but a low incidence in the newest wood 0.5 cm from the cambial layer (Fig. 2). Areas of high hydraulic conductivity generally had the smallest number of plugs in vessel cross section and areas of low conductivity had the largest number of plugs (Figs. 2 and 3). Amorphous plugs outnumbered the filamentous type (Fig. 2). Filamentous plugs were found mainly at the centers of the wood disks in blighted trees as in healthy trees. No substantial differences in the distribution of vessel plugs were seen among blighted trees on different rootstocks. The number of vessels in a low-power microscope field changed with the different tissues studied (eg, trunks had fewer vessels per field than did small roots or small stems). Data in Fig. 2 on number of plugs seen at any point should therefore be compared only with data from corresponding points on other trees.

Mean hydraulic conductivity of twigs from healthy trees was almost identical to the hydraulic conductivity of twigs from trees with blight (5). Despite the similarity in conductivity there was a difference in incidence of vessel plugging between twigs from healthy and blighted trees (Table 1). Plugs were practically absent from twigs from healthy trees but, although irregularly distributed, plugs were present in some twigs from trees with blight. Average hydraulic conductivity in pencil-sized roots of healthy trees was higher than that in the corresponding roots of trees with blight (5) with considerable overlapping of individual measurements. Plugs were absent from the vessels of roots from healthy trees and present, in irregular numbers, in most roots from trees with blight. Generally the most highly conductive stem and root segments had the lowest incidence of vessel plugs (Table 1).

DISCUSSION

The plugging of vessels in the inner wood of the trunk, large branches, and large roots of trees with citrus blight was associated with the reduction in hydraulic conductivity found in blight disease. The relative absence of plugging and comparatively high hydraulic conductivity of a layer of wood about 1 cm thick inside the cambium of blighted trees permits good water movement in a 1-cm-wide cylinder and may be associated with the fact that trees with blight continue to live almost indefinitely after becoming

TABLE 1. Hydraulic conductivity and number of vessel plugs in 1.25 cm lengths of twigs and pencil-sized roots of healthy and blighted trees

Tree			Twigs						Pencil-sized roots					
			Most conductive[a]			Least conductive[a]			Most conductive[a]			Least conductive[a]		
	Condition	Rootstock	Conductivity (ml/cm² min)	Plug type (no.) Filamentous	Plug type (no.) Amorphous	Conductivity (ml/cm² min)	Plug type (no.) Filamentous	Plug type (no.) Amorphous	Conductivity (ml/cm² min)	Plug type (no.) Filamentous	Plug type (no.) Amorphous	Conductivity (ml/cm² min)	Plug type (no.) Filamentous	Plug type (no.) Amorphous
A	Healthy	Cleo	115	3	1	69	0	2	986	0	0	329	0	0
D		Sweet	113	0	0	55	0	0	1,800	0	0	792	0	0
G		Rough	120	0	0	64	0	0	1,137	0	0	333	0	0
B	Blighted	Cleo 1	154	34	44	37	0	66	1,500	0	4	62	13	50
C		Cleo 2	98	0	4	74	0	0	1,137	0	7	110	8	104
E		Sweet 1	120	0	7	60	0	2	1,661	0	0	304	0	13
F		Sweet 2	177	0	0	57	0	0	332	0	7	58	1	91
H		Rough 1	143	0	0	40	5	9	750	0	36	275	1	6
I		Rough 2	129	0	0	71	0	0	1,661	0	0	568	1	8

[a]Most and least conductive of 10 samples from each tree.

diseased. The high incidence of vessel plugging and low conductivity of wood more than 1 cm deep in blighted trees appears to be a feature of the decline condition. This condition in blighted trees must be contrasted to the high conductivity and low incidence of vessel plugs across the trunk disk (except near the pith) found in healthy trees. High conductivity across most of the diameter of the trunk seems to be characteristic of healthy citrus trees generally.

Plugs seen in vessels of trunks, limbs, and large roots of blighted trees were almost exclusively of the amorphous type. This suggests that amorphous plugs are the characteristic vessel blockage

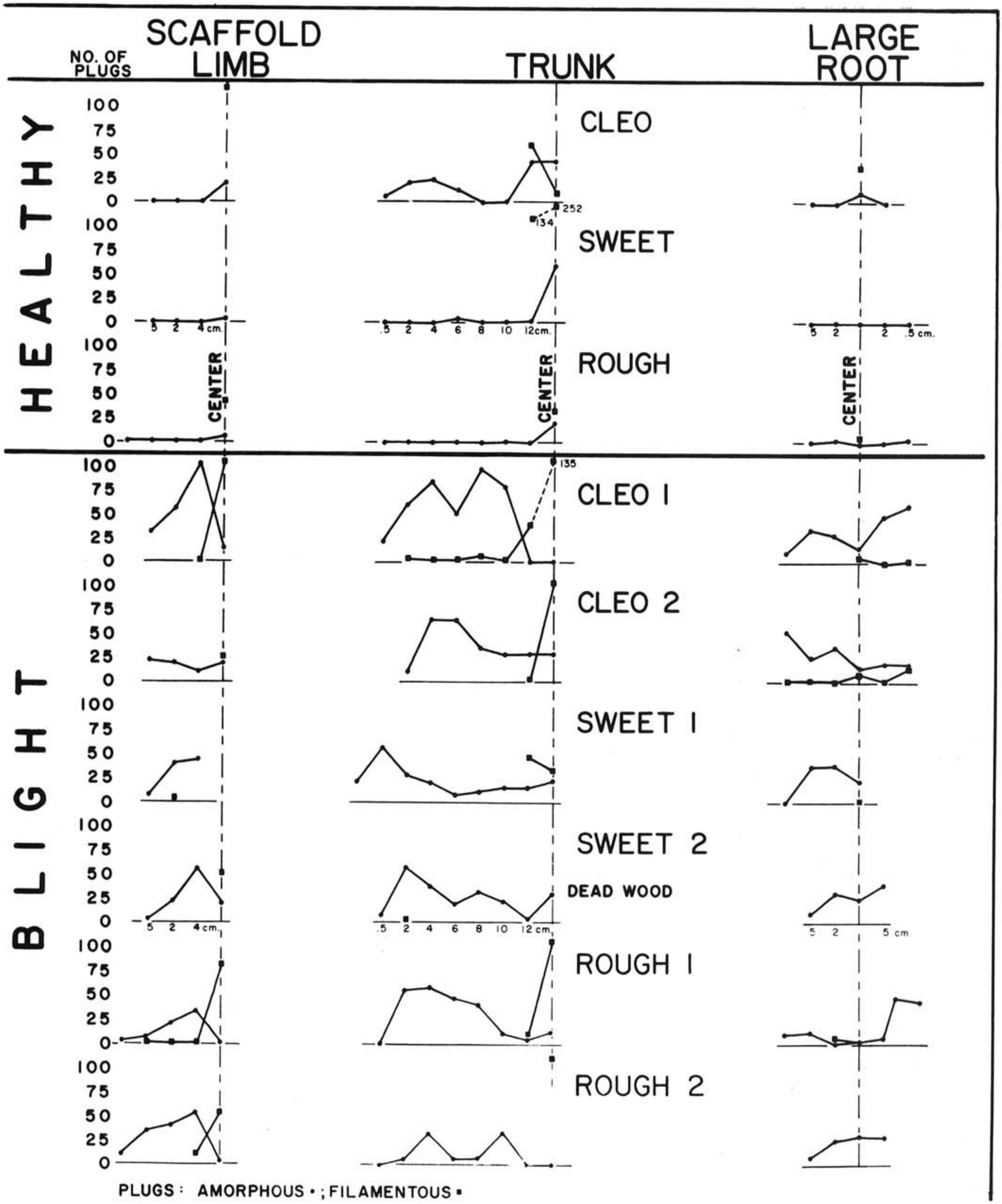

Fig. 2. Distribution of xylem vessel plugs in healthy and blighted trees. Numbers of amorphous and filamentous plugs seen in five ×100 microscope fields at 2-cm intervals along the diameter of cross sections of scaffold limbs, trunks, and large roots. Vessel plugging is minimal in healthy trees except near the pith at the center. In trees with blight, amorphous plugs are the most numerous, by far, but filamentous plugs predominate near the pith of scaffold limbs and trunks. Incidence of plugs is low in the conductive outer rim of the wood disks of blighted trees.

structures of trees with blight. VanderMolen et al (10), studying small roots, saw the correlations between the incidence of amorphous plugging and severity of disease, but did not look elsewhere in the trees. Nemec et al (8) reported that amorphous plugs were almost three times as numerous as filamentous plugs in new and intermediate trunk wood of blighted trees, but did not comment on the significance of the amorphous plugs. Much of the previous work on vessel plugging in trees with blight has concentrated on filamentous plugs (1,2,7,9). Our data (Fig. 2) indicates that the distribution and number of filamentous plugs is very similar in healthy trees and in trees with blight, but high counts of amorphous plugs are associated with blighted trees only. An

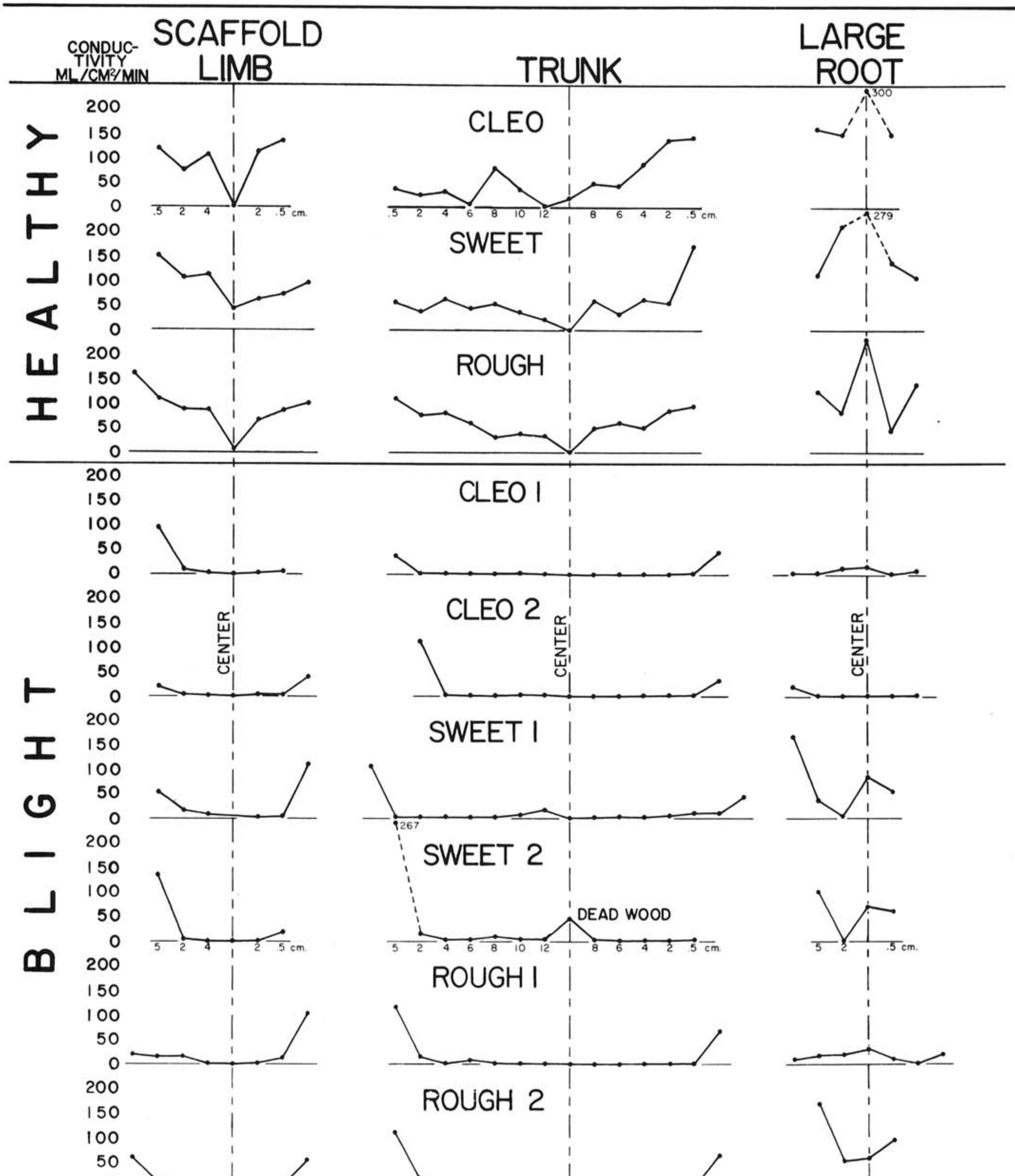

Fig. 3. Hydraulic conductivity (milliliters per square centimeter per minute) of healthy trees and trees with citrus blight on three rootstocks. Conductivity was determined by measuring movement of water through wood pegs 0.8 cm in diameter and 1.25 cm long into a chamber that was 75% evacuated. Pegs were punched out with a special tool at 2-cm intervals along the diameter of cross section disks from scaffold limbs, trunks, and large roots.

indication of earlier neglect of amorphous plugs is the fact that the photos of amorphous plugs in Fig. 1 are the first to be published.

In the work that is reported here, the anatomical changes that characterize the blight condition were found to be located in the trunk, the major limbs, and large roots rather than in small branches and small roots. The importance of blockage of water movement in the trunk, major limbs, and large roots to blight is reflected also in the data on hydraulic conductivity (Fig. 3). These findings confirmed the statement of Young (12), reporting the results of his waterflow studies that "the major areas in blighted trees where massive reductions in water movement occur are the trunks and scaffold limbs." The nine trees used in this investigation are, admittedly, a very small sample of the millions of trees in Florida groves, but the blighted and healthy trees that were studied appear to be fully representative individuals. It remains for further studies and experience with blight disease to determine whether conclusions stated in this paper are confirmed.

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