Structural Comparison of Xylem Occlusions in the Trunks of Citrus Trees with Blight and Other Decline Diseases

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ABSTRACT

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Xylem occlusions were compared in citrus trees with blight (cause unknown); tristeza (caused by citrus tristeza virus); psorosis A and concave gum (caused by graft-transmissible agents); stubborn (caused by *Spiroplasma citri*); Phytophthora foot rot; and citrus slump (caused by *Pratylenchus coffeae*). Filamentous plugs observed in trees with citrus blight were also present in the xylem vessels of trees affected with stubborn, citrus slump, tristeza, and foot rot as well as in many healthy trees. Amorphous plugs were more numerous in trees with blight than in healthy trees. A similar type of plug was observed by light microscopy in trees with psorosis and concave gum. However, these amorphous-like plugs in trees with psorosis and concave gum could be differentiated from those present in trees with citrus blight by scanning and transmission electron microscopy. Thus, amorphous plugs are characteristic of blight trees and can be used for diagnosis along with zinc accumulation and reduced water uptake if care is taken to separate them from the amorphous-like occlusions that form in plants affected by other diseases.

Additional key words: *Citrus paradisi*, *C. sinensis*, young tree decline.

Citrus blight is a decline disease of unknown etiology. Some other diseases produce canopy decline symptoms that often resemble those of blight. Therefore, blight cannot be diagnosed from canopy symptoms alone (1). Blight is characterized by reduced water conductivity of the trunk and root xylem (8,11,20), elevated zinc levels of the trunk wood (14,19), and the presence of occlusions in xylem vessels (4,6,8,10,15). Two types of occlusions or plugging materials described as filamentous or fibrous plugs (6,8) and amorphous or gum plugs (8,11) have been observed in the xylem of blight-affected trees. Filamentous plugs consist of a mass of fine fibers (0.2–0.7 μm in diameter) and are usually found at vessel end walls. Amorphous plugs appear to be solid and may completely block the vessel lumen.

Childs and Carlyle (6) reported the presence of filamentous plugs in the xylem of roots of blight-affected trees and concluded they were the mycelia of *Physoderma* sp. Vandermolen (15) found that the ultrastructure of the filamentous plugs in roots from blight-affected trees did not resemble that of fungi, actinomycetes, bacteria, mycoplasmas, or other organisms.

Nemec and Kopp (12) reported that lipid (filamentous) plugs were prevalent in healthy as well as blight-affected trees and that they appeared to be the likely obstruction to water movement. Childs (5) suggested that the blockage in the xylem of blight-affected trees was caused by fibrous plugs. Nemec et al (11) studied the two types of plugging materials in the xylem and found that the number of filamentous plugs did not differ in blight-affected and healthy trees but that a higher percentage of vessels in the roots of blight-affected trees contained amorphous and filamentous plugs. They concluded that amorphous plugs in the trunk wood of blight-affected trees appeared to be the main resistance to water movement in vessels along the cambium. In 1983, Cohen et al (8) studied the two types of occlusions and found that amorphous plugs were associated with a reduction in water conductivity in the trunk. Brlansky et al (3) showed that as the number of amorphous plugs in trunk wood increased, water uptake was reduced and the canopy decline progressed. No such association existed with the filamentous plugs.

TABLE 1. Characteristics of healthy and declining citrus trees

Location/ cultivar[a]	Tree diagnosis	No. of trees	Zn (μg/g)	Water uptake (ml/sec)	Average number of plugs 200 vessels		
					Filamentous-like	Amorphous-like	Total
Florida	Healthy	5	5.6	1.15	0.90	0.70	1.60
SwO/RL	Blight	5	10.9*[b]	0.00*	0.50 n.s.	23.20*	23.70*
Florida	Healthy	6	0.62	0.68	7.80	0.50	8.33
G/SO	Blight	6	2.29*	0.03*	6.10 n.s.	26.30*	32.50*
California	Healthy	4	1.47	0.43	2.25	0.00	2.25
SwO/T	Citrus stubborn	4	0.94 n.s.	0.81 n.s.	0.50 n.s.	0.00 n.s.	0.50 n.s.
California	Healthy	5	1.42	0.92	0.60	0.00	0.60
SwO/various	Concave gum	3	1.12 n.s.	0.51 n.s.	7.00 n.s.	8.00 n.s.	15.00 n.s.
rootstocks	Psorosis A	4	1.19 n.s.	0.39 n.s.	48.00*	26.25 n.s.	74.25 n.s.
Lake Mattie	Healthy	6	2.02	0.66	0.50	0.00	0.50
SwO/RL	Citrus slump	6	3.85*	0.62 n.s.	2.33 n.s.	0.00 n.s.	2.33 n.s.
Turner	Healthy	5	0.37	0.92	0.00	0.00	0.00
SwO/SO	Tristeza	5	0.40 n.s.	0.60 n.s.	0.00 n.s.	0.00 n.s.	0.00 n.s.
Kelly	Healthy	5	0.70	1.87	0.00	0.00	0.00
SwO/SO	Foot rot	5	0.45 n.s.	1.00 n.s.	1.00 n.s.	0.00 n.s.	1.00 n.s.

[a] SwO = sweet orange (*Citrus sinensis* L.), G = grapefruit (*C. paradisi* L.), RL = rough lemon (*C. jambhiri* Lush), T = troyer citrange (*Poncirus trifoliata* L. Raf. × *C. sinensis* L.), SO = sour orange (*C. aurantium* L.).

[b] Means significantly different from the corresponding healthy controls according to Fisher's least significant difference test at $P = 0.05$. The abbreviation n.s. stands for "not significant."

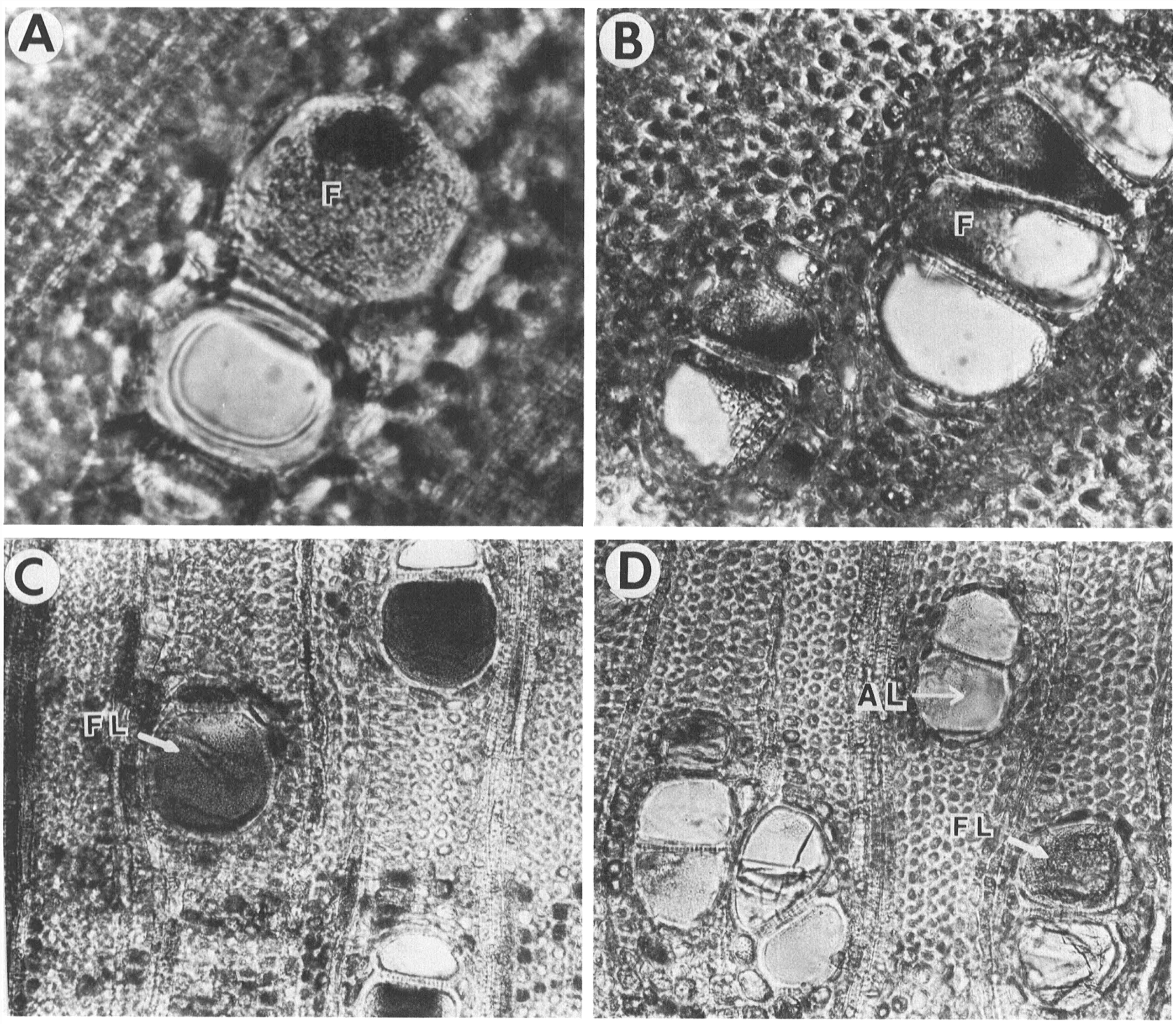

Fig. 1. Light micrographs of filamentous and filamentous-like plugging materials in trunk wood xylem vessels of decline-affected citrus trees. **A,** Filamentous plug (F) in xylem of blight-affected citrus (×276). **B,** Filamentous plugging (F) in xylem of stubborn-affected citrus (×276). **C,** Brown-colored filamentous-like plugs (FL) in the xylem vessels of trees affected with psorosis A (×150). **D,** Filamentous-like (FL) and gold colored amorphous-like (AL) plugs in the xylem vessels of trees affected with psorosis A (×150).

Vascular plugging has been reported in psorosis-affected citrus (2,18) and was described as gum plugs due to wound gumming (13). A gum substance similar to that seen in psorosis has also been described in citrus trees with concave gum (17). Water uptake tests and zinc accumulation in the trunk wood or bark have been used to separate blight-affected citrus trees from trees with other decline problems (1,7,9,19).

The possible presence and extent of vascular plugging has not been examined for many citrus decline diseases. The purpose of this study was to make a cytological comparison of the vascular occlusions in the trunks of blight-affected trees with those in trees affected by other decline diseases.

MATERIALS AND METHODS

Samples. Sweet orange trees (*Citrus sinensis* (L.) Osbeck) on rough lemon rootstock (*C. jambhiri* Lush) were selected from a grove in central Florida and grapefruit trees (*C. paradisi* Macf.) on sour orange rootstock (*C. aurantium* L.) were chosen from a grove in southeastern Florida. Trees in all stages of blight and healthy trees were selected on the basis of symptomatology, syringe water-injection test (9), and zinc analysis of the trunk wood (19).

Sweet orange trees on sour orange rootstock infected with citrus tristeza virus (CTV) were selected on the basis of stunting, decline, and typical "honeycombing" of the bark below the bud union. Trees were indexed on Mexican lime (*C. aurantifolia* (Christm.) Swingle) indicator seedlings to confirm the presence of CTV. Sweet orange trees on sour orange rootstock affected by foot rot (*Phytophthora* sp.) were sampled. The trees were in decline and had typical bark lesions above the bud union. Citrus slump-affected sweet orange trees on rough lemon rootstock were sampled in a grove in central Florida. Samples from psorosis A-, concave gum-, and stubborn-affected citrus trees were collected from the experimental farm at the University of California, Riverside.

Diagnostic tests. Trees were tested for blight by the syringe injection test for water conductivity (9) and for zinc accumulation in the trunk wood (19). Core samples of the trunk wood 6-cm long were taken with a Hagloff 5-mm-diameter increment borer just above the hole for the syringe injection test. After removal, the cores were immediately fixed for 8–15 hr in a 3% glutaraldehyde solution in 0.066 M sodium-potassium phosphate buffer, pH 6.8, and washed in the same buffer. The 2–3-cm portion of each core as measured from the cambium was cut out and divided into three equal pieces.

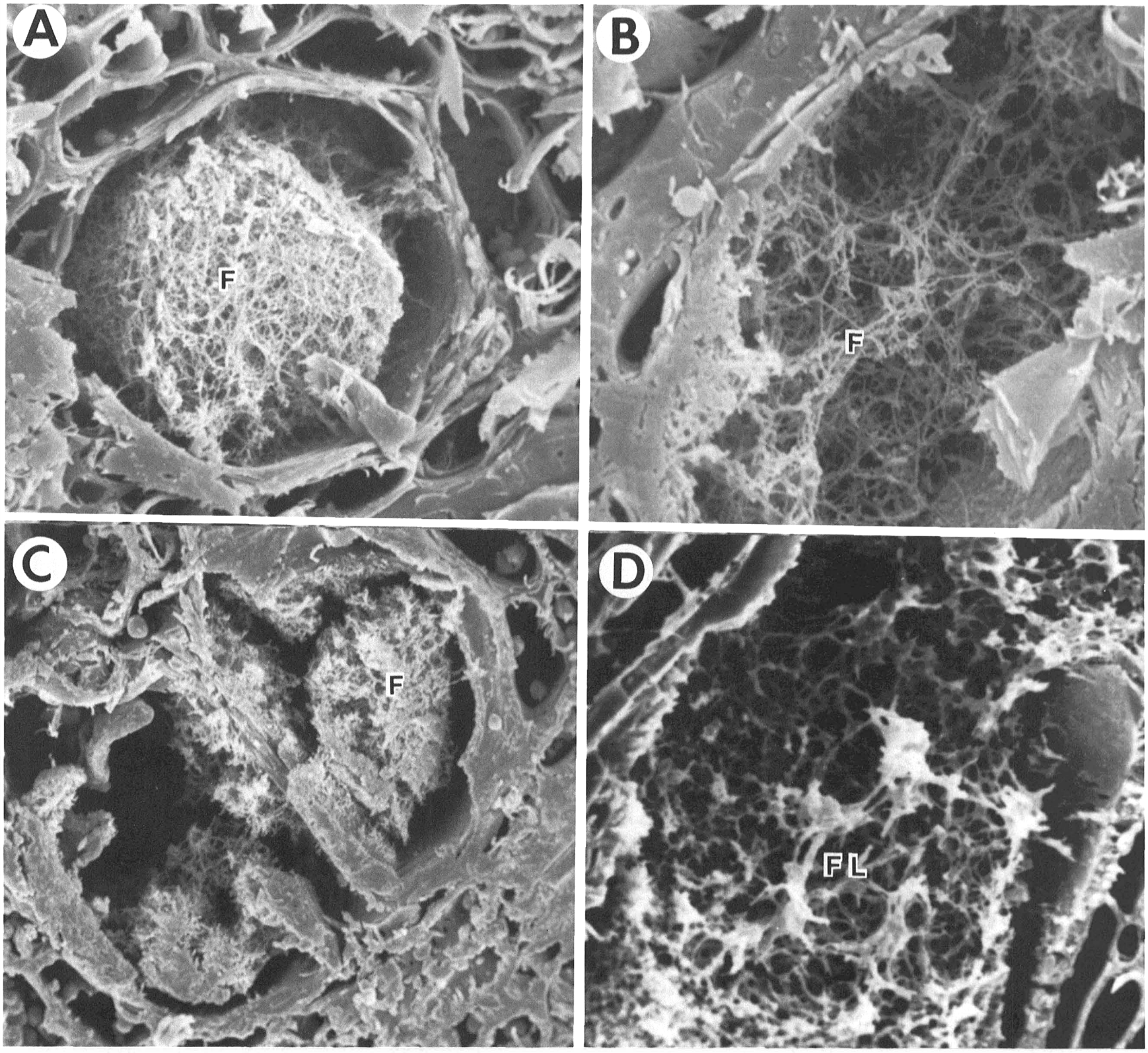

Fig. 2. Scanning electron micrographs of the filamentous and filamentous-like plugging in the trunk wood xylem of decline-affected citrus trees. **A,** Filamentous plugging (F) in the xylem of blight-affected citrus (×940). **B,** Filamentous plugging (F) in the xylem of healthy citrus (×860). **C,** Filamentous plugging (F) in a xylem vessel of stubborn-affected citrus (×660). **D,** Filamentous-like plugging (FL) in psorosis A-affected citrus (×800).

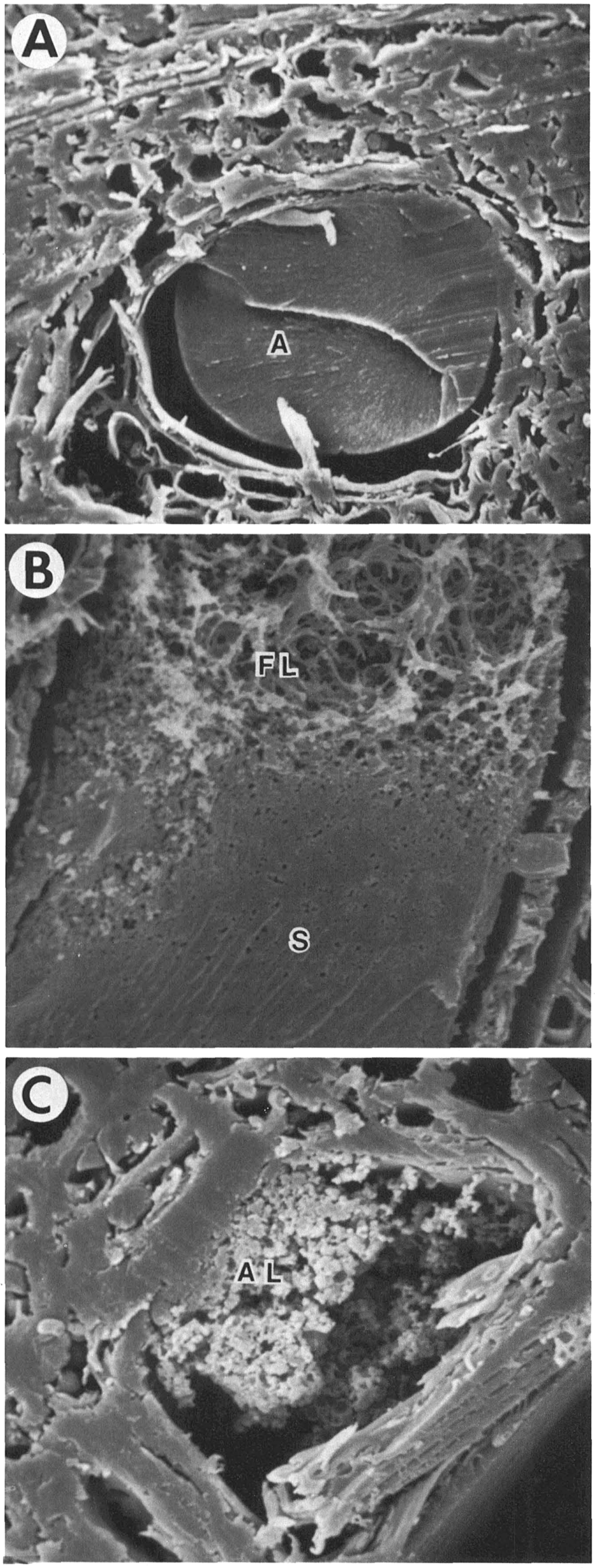

Fig. 3. Scanning electron microscopy of the amorphous or amorphous-like plugging material in declining citrus trees. **A,** Amorphous plug (A) in the xylem vessel in a blight-affected citrus tree (×440). **B,** Plugging material in the xylem of a psorosis A-affected tree showing the coalescence of the filamentous-like (FL) material into a compressed or compacted solid form (S) (×860). **C,** Amorphous-like plugging (AL) material in the xylem of concave gum-affected trees (×940).

Light and electron microscopy. For light microscopy, longitudinal and transverse sections, 30–40 μm thick, were cut from one piece on a A-O Spencer model 860 sliding microtome and viewed with an Olympus BH-2 light microscope. The number of brown filamentous and yellow or gold amorphous plugs were counted in 200 randomly selected xylem vessels.

The remaining two portions of the core were prepared for scanning (SEM) and transmission (TEM) electron microscopy. Samples were dehydrated in a 30–100% acetone series for 20 min per step. For SEM, samples were transferred to an acetone:freon series (2:1 and 1:2) for 20 min each and then into two changes of 100% freon and critical-point dried in a Bomar Critical-Point Drier (The Bomar Co., Tacoma, WA). The specimens were mounted on SEM stubs, sputter coated with 100 Å of gold-palladium, and viewed in a JEOL JSM 35 scanning electron microscope. For TEM, specimens were placed in Spurr's (firm formulation) medium:acetone (1:2) for 12 hr, transferred to a 2:1 mixture for 12 hr, and then into 100% Spurr's medium for 12 hr. The samples were then polymerized in Spurr's medium at 70 C. Ultrathin sections were made on a Huxley LKB ultramicrotome (LKB Instruments, Rockville, MD), mounted on grids, stained with uranyl acetate and lead citrate, and viewed with a Philips 201 transmission electron microscope.

RESULTS

Significantly reduced water uptake rates together with elevated zinc levels in the trunk wood occurred only on trees with blight (Table 1). Water uptake rates of stunted sweet orange trees infected by CTV were slightly, but not significantly, lower than those of healthy trees. Almost no water uptake occurred in trees with psorosis or concave gum if the injections were performed in the areas of bark scaling or of gum pockets, but these trunks did not have elevated levels of zinc. Zinc levels in trees affected by *P. coffeae* were significantly higher than those of the healthy controls.

With the light microscope, filamentous and amorphous plugs were found in the xylem vessels of the trunk wood in all blight-affected trees (Figs. 1A and 4A). Filamentous plugs also were present in trees affected by citrus stubborn (Fig. 1B), psorosis A, concave gum, foot rot, and slump. Except for psorosis A-affected trees, no significant differences were found in the number of filamentous plugs between healthy trees and trees affected by these declines (Table 1). In each case, the filamentous plugs were brown and threadlike, and similar to those present in trees with citrus blight (Fig. 1). Some morphological differences were noted in the filamentous plugging found in psorosis A samples. The filaments or strands were often found in conjunction with a yellow-gold plugging material (Fig. 1D) as previously described (2,16).

The filamentous plugs in healthy, and in blight-, foot rot-, slump-, and stubborn-affected trees all appeared similar under scanning electron microscopy (SEM) (Fig. 2A, B, and C). Strans of thread-like material were present in the vessel lumens and along the vessel walls.

The filamentous-like plugging material found in the xylem of psorosis A-affected trees appeared to be structurally different than the material described in healthy and blight-affected trees. In scanning electron micrographs (Fig. 3B), it was less threadlike and appeared to be part of an amorphous type plug. The amorphous part of this plug did not appear to be as solid as it appeared to be under light microscopy or as solid as the amorphous plugs found in blight-affected trees (Fig. 3A).

Amorphous-like plugs from concave gum-affected trees were neither filamentous nor amorphous in structure (Fig. 3C) when viewed with SEM. The texture of the plugs was particulate, consisting of small darkly stained pieces of material. With the light microscope, amorphous blight plugs, concave gum plugs, and the solid portion of the psorosis A plugs all appeared yellow to gold and were solid (Fig. 4A,C, and E).

When ultrathin sections of these three types of occlusions were viewed with TEM, differences in their structures were seen (Fig. 4B,D, and F). The amorphous occlusions from blight-affected trees were dense or nonporous and almost completely filled the vessel

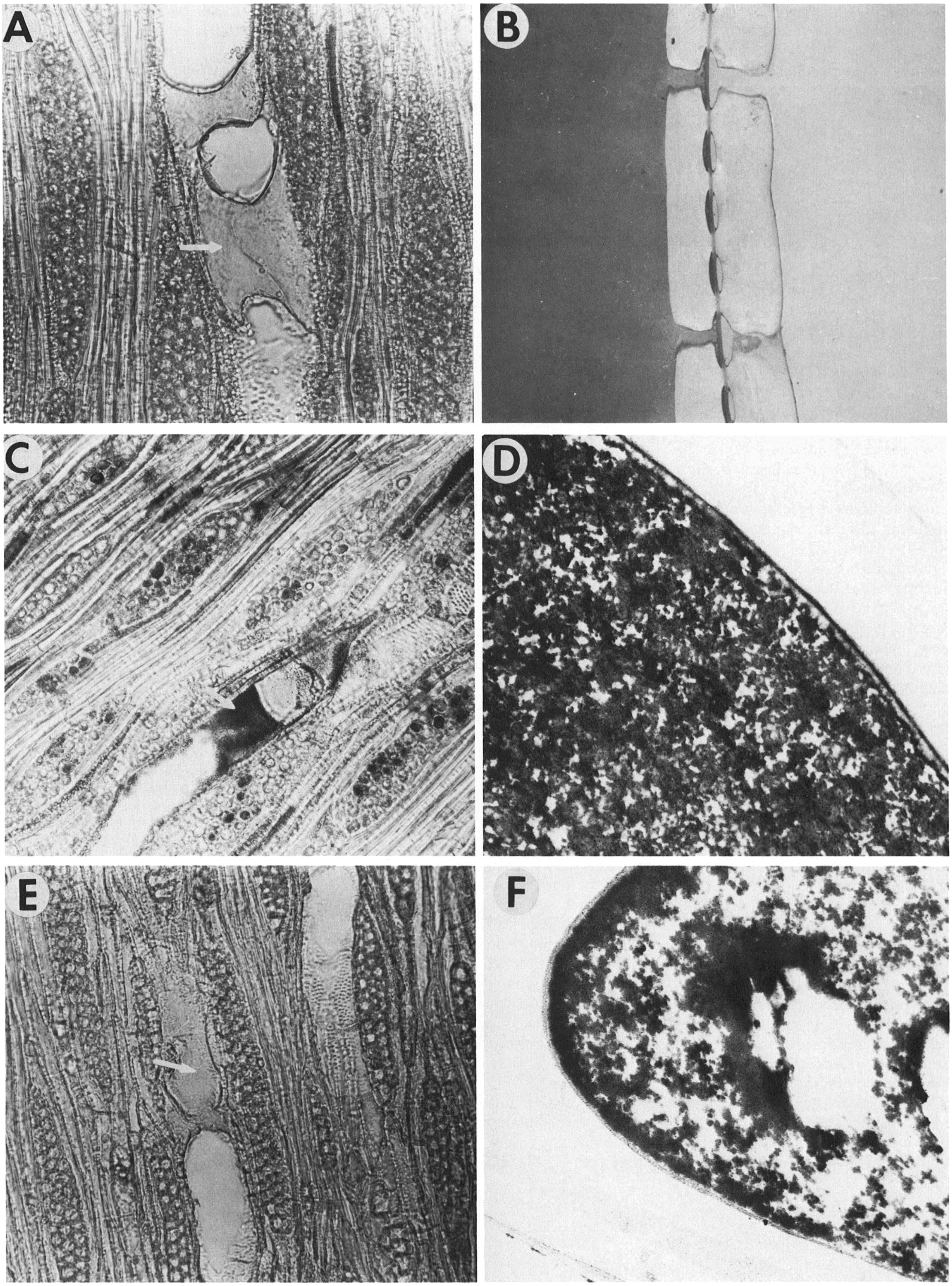

Fig. 4. Light and transmission electron microscopy of amorphous and amorphous-like xylem plugs in blight-, psorosis A-, and concave gum-affected citrus trees. **A,** Light micrograph of an amorphous xylem plug (arrow) in a blight-affected tree (×150). **B,** Transmission electron micrograph of an amorphous plug in a blight-affected tree showing the density of the plug and apparent filling of the pits (×3,050). **C,** Light micrograph of the combination filamentous-amorphous plug (arrow) in a psorosis A-affected tree (×150). **D,** Transmission electron micrograph of the plugging in the psorosis A-affected tree showing the density of the plug as well as its particulate nature (×7,000). **E,** Light micrograph of the amorphous-like material (arrow) from a concave gum-affected tree (×150). **F,** Transmission electron micrograph of the concave gum plugging showing the density of the material near the vessel wall and the porous nature in the lumen of the vessel (×4,650).

lumen. This plugging material also occurred in pits. The psorosis A plugging material was composed of a compacted mass of weblike strands. The material was very dense along the vessel wall but more particulate in the vessel lumen (Fig. 4D). The fibrous nature of the occlusion seen in scanning electron micrographs (Fig. 3B) was often seen in some of the sectioned vessels. The particulate nature of the concave gum plugs was revealed in ultrathin sections (Fig. 4F). The plugs appeared nonporous along the vessel wall, but they were very particulate or porous in the vessel lumen. At times, pits were filled with the densely staining material.

DISCUSSION

The two main tests commonly used for diagnosis of citrus blight, reduction in water uptake, and zinc accumulation in the trunk wood (1,7,9,19) were specific for citrus blight. In all the other decline diseases in this study, no significant differences were found between the water uptake rates in diseased and healthy trees. However, water uptake was reduced if injections were made into lesion areas where gum is abundant in psorosis A-affected trees or into concavities where concave gum is present. Zinc content of the trunk wood was significantly higher only in blighted and trees affected by *P. coffeae*; the reason for the high zinc levels in the latter is unknown. However, blight was prevalent in the grove area and some trees could have been in the early stages of blight.

Significant differences in the numbers of plugs between diseased and healthy trees were found only with blight (Table 1). Filamentous plugs commonly associated with blight-affected citrus trees were present in healthy trees as well as in trees with other declines. Obviously, the filamentous plugging previously associated with blight-affected trees (5,6) is not specific for this disease. The filamentous plugging in psorosis A-affected trees was different from that seen in trees affected by blight or other declines. These plugs were similar to the filamentous blight plugs when viewed by light microscopy, but were associated with golden or amber colored amorphous-like plugs (Fig. 1C and D). When observed by SEM, this filamentous plugging was more strandlike and it appeared to condense into a dense, solid-appearing amorphous-like occlusion. The more particulate strandlike texture was further revealed with TEM and showed the lack of similarity between these plugs and the filamentous and amorphous plugs.

The amorphous-like plugs in concave gum-affected trees also appeared similar to amorphous plugs of blight-affected trees when viewed by light microscopy; however, differences were apparent when these were viewed with SEM. Concave gum plugs were particulate in the center, but the edge of the plug near the vessel wall was dense and solid. In ultrathin sections, the plugs appeared to be solid near the vessel wall but were more particulate in the vessel lumen.

Cytochemical tests may reveal some of the differences or similarities among these xylem occulsions. However, only when the exact chemical composition is determined by analytical means can their chemical nature be compared.

No bacteria or fungi were observed with either TEM or SEM in any of the samples. The amorphous plugs associated with blight-affected trees appear to be responsible for reduced water uptake, and are consistently associated with the disease. Amorphous plugs are characteristic of citrus blight and together with zinc accumulation in trunk wood and reduced water uptake can be used as a diagnostic criterion. However, care must be taken since plugs which superficially resemble amorphous-like plugs occur in other trees affected by other diseases such as concave gum.

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