Occurrence of Xylem Occlusions in Diseased Citrus Plants Under Greenhouse Conditions

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ABSTRACT. An investigation was conducted to establish the nature and incidence of amorphous and fibrous occlusions in the xylem of diseased and healthy appearing citrus trees in the greenhouse. Trees that were examined included 8-yr old Valencia orange plants on rough lemon rootstocks as well as 20-month-old Valencia and rough lemon seedlings exhibiting either *Fusarium* or *Phytophthora* root rot. Cross sections of roots, stems and branches were cut with a cryostat and prepared for scanning electron microscopy and light microscopy. Amorphous occlusions were similar to those associated with citrus blight. In some instances more than ten amorphous occlusions were observed per 200 xylem vessels. These results may have important implications pertaining to the etiology of citrus blight.

Vascular occlusions occur in citrus affected by concave gum (4, 21) psorosis (3, 4, 22) and citrus blight (4, 6, 12, 15, 16, 20) a disease of unknown etiology. An increase in numbers of xylem occlusions of the "resinous type" was recorded in citrus plants affected by *Fusarium solani* or dihydrofusarobin and isomarticin extracted from cultures of the fungus (13, 14).

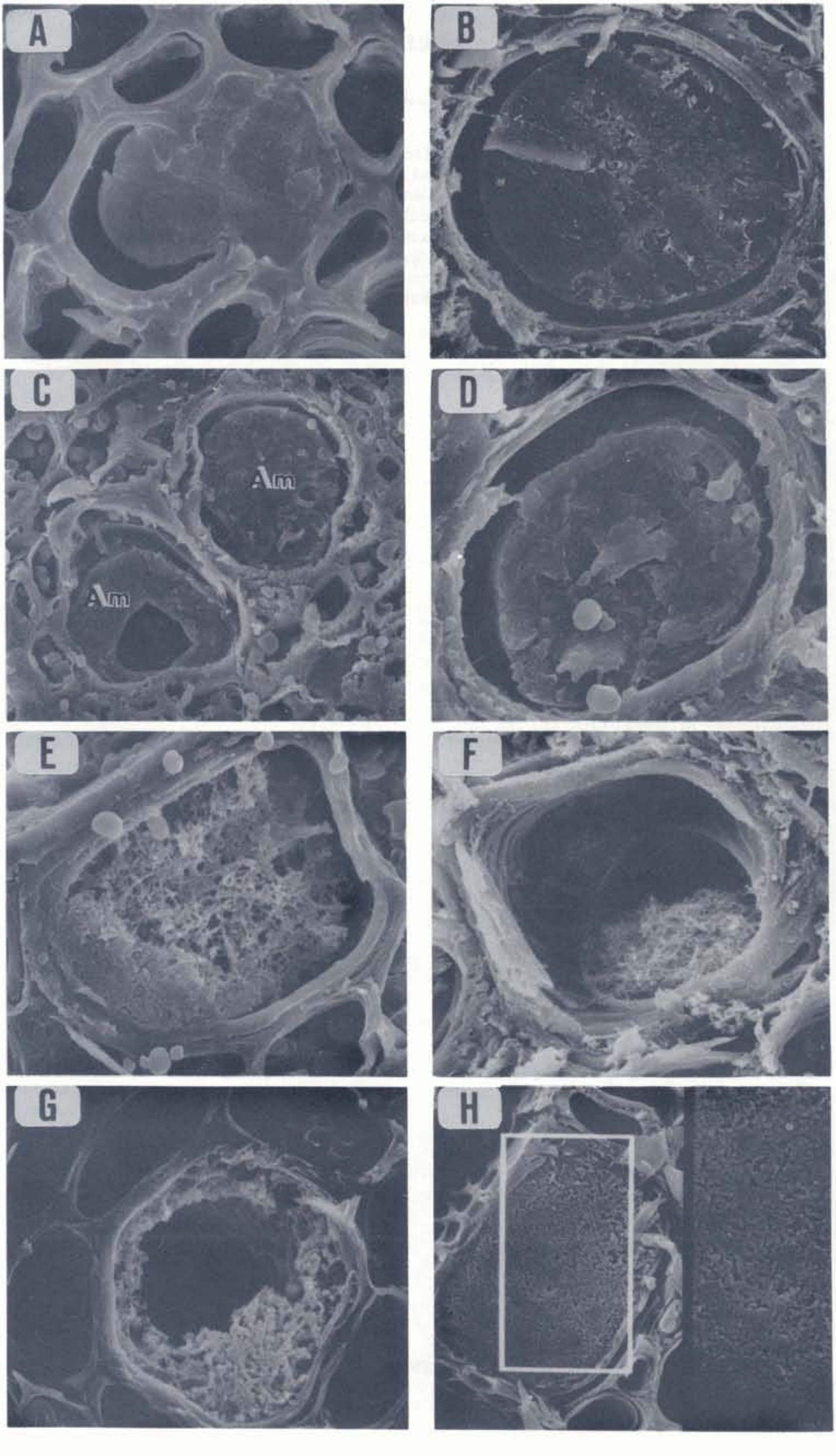
Xylem occlusions observed in blight-affected and healthy looking citrus trees were either amorphous (gum) (4, 6, 15, 20) or fibrous (filamentous or lipid) (16, 12, 16, 20). Both types occur in the roots, trunks and twigs of diseased and healthy appearing trees (4, 6, 15, 20), although a greater number of occlusions are present in trees affected by blight (6, 15, 18, 20). Amorphous occlusions are associated with reduced water uptake (5, 6, 18) and increasing numbers of these occlusions correlate with increasingly severe blight symptoms (5, 20). Cohen *et al.* (6) and Brlansky *et al.* (4) considered amorphous occlusions to be characteristic of citrus blight. Brlansky *et al.* (4) reported that amorphous occlusions in trees with psorosis and concave gum differed from those in trees with blight in that they had amorphous as well as filamentous characteristics. They also observed that the filamentous occlusions in psorosis-affected trees were different in structure from those seen in trees with blight or healthy trees. The chemical nature of amorphous and filamentous occlusions in blight-affected trees was studied by various researchers (2, 12, 15, 16, 20).

The present study reports on the nature and incidence of xylem occlusions in healthy citrus plants and in plants with root rot in the greenhouse. Occlusions observed in these plants are also compared with those present in blight-affected trees in Florida.

MATERIALS AND METHODS

A total of 43 healthy and diseased 20-month-old Valencia sweet orange and rough lemon seedlings as well as four 8-yr-old Valencia sweet orange trees on rough lemon rootstock, reared in the greenhouse, were investigated. For comparative light and scanning electron microscopy (SEM), fixed root samples were also obtained from a blight-affected tree, Valencia sweet orange on Carrizo citrange in Florida (courtesy Dr. L. J. Marais, S. A. Cooperative Citrus Exchange). This tree was previously tested by means of the water-uptake and zinc accumulation tests in addition to the observation of high numbers of amorphous plugs in the xylem.

Healthy and diseased seedlings were selected from an inoculation experiment in which plants were inoculated with a suspension of *Phytophthora nicotianae* var.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parasitica (Dastur) Waterhouse mycelium and zoosporangia applied to the potting medium, 9 months before the present investigation started. At the time of sampling, diseased seedlings exhibited leaf yellowing.

Isolations and root rot assessment. Selected seedlings were removed from their pots, their roots rinsed under running tap water and root rot severity estimated according to a scale 0-4 with 0 = no root rot and 1-4 = increasingly severe root rot. The potting medium of each plant was assayed for the presence of *Phytophthora* spp. by means of the citrus leaf baiting technique (8). To determine the presence of *Phytophthora* spp. and *Fusarium* spp. in the roots, subsamples of the primary and feeder roots from each plant were cut in 4-mm long segments, surface sterilized for 60 sec in 1.5% sodium hypochlorite, rinsed in sterile water and plated on agar medium. Potato dextrose agar supplemented with 125 ml/liter chloramphenicol and the medium of Tsao & Guy (19) was used for isolating *F. solani* and *Phytophthora* spp. respectively. After incubation for 6 days at 25C in the dark, plates were examined for growth of *Fusarium* spp. and *Phytophthora* spp. Feeder roots and 4-mm diameter roots of the 8-yr-old sweet orange on rough lemon trees were sampled and treated as described for the seedlings.

Light and electron microscopy. Five millimeter long segments were cut from the primary roots (2 cm below soil level) and stems (2 cm above soil level) of each sweet orange and rough lemon seedling and from 4 mm diameter roots and twigs of each sweet orange tree on rough lemon rootstock. Ten cross sections, 25 μm thick were cut from each segment on a Reichert-Jung model 856 cryostat microtome. These were mounted in lactophenol and examined under the light microscope. The number of amorphous and filamentous occlusions in the xylem was recorded at 100X magnification. Representative samples of remaining portions of segments were prepared for scanning electron microscopy. Root samples from the blight-affected tree had been fixed in 3% glutaraldehyde and subsequently kept in phosphate buffer saline (PBS) containing sodium azide.

Samples for SEM were fixed by exposure to 2% osmium tetraoxide vapour in a dessicator for 48 hr (17). The samples were subsequently air dried in a fume extraction cabinet for 48 hr, mounted on standard copper stubs, sputter-coated with gold and examined with a Hitachi S-450 scanning electron microscope.

RESULTS

Incidence of root pathogens and extent of root rot. *P. nicotianae* var. *parasitica* was consistently isolated from potting medium of plants with root rot, while *F. solani* was isolated from the roots of diseased as well as healthy appearing plants (Table 1). Only three plants had *Fusarium*-associated root rot in the absence of *Phytophthora*. Because the roots of diseased plants were in an advanced stage of rot, *P. nicotianae* var.

Fig. 1. Scanning electron micrographs of xylem occlusions in roots of citrus plants affected by *Phytophthora* root rot (PRR) in the greenhouse and by blight in Florida. A) Amorphous occlusion in PRR-affected Valencia orange seedling (X2,000); B) Amorphous occlusion in blight-affected tree (X1,000). C) Amorphous occlusions (Am) completely and partly occluding vessel in PRR-affected Valencia orange seedling (X1,000); D) Amorphous occlusion in PRR-affected Valencia orange/rough lemon rootstock (X2,400); E) Filamentous occlusion in PRR-affected Valencia orange/rough lemon rootstock (X2,000); F) Filamentous occlusion in blight affected tree (X2,000); G) Filamentous occlusion in PRR-affected Valencia orange seedling (X2,000); H) Porous occlusion with amorphous and filamentous characteristics in PRR-affected Valencia orange seedling (X550, inset X1,100).

TABLE 1

SUMMARY OF ROOT ROT SEVERITY, PRESENCE OF *PHYTOPHTHORA NICOTIANAE* VAR. *PARASITICA* AND *FUSARIUM SOLANI*, AND INCIDENCE OF XYLEM OCCLUSIONS IN 20 MO-OLD SWEET ORANGE (SWO) AND ROUGH LEMON (RL) SEEDLINGS AND 8 YR-OLD SWO/RL TREES UNDER GREENHOUSE CONDITIONS

					Average no. of occlusions per cross section[w]					
			Root pathogen		Roots[v]			Stems[u]		
Plant	No. of plants	Root rot severity[z]	*Phytophthora*[y]	*Fusarium*[x]	Amorph.	Filam.	Total	Amorph.	Filam.	Total
SWO seedl.	7	2.7	+	+	10.5*	41.3*	51.8*	4.7*	8.7*	13.4
SWO seedl.	7	0	–	+	0.9	1.4	2.3	0.01	0.02	0.03
RL seedl.	13	3.7	+	+	1.0*†	5.2*	6.2*	0.3*†	0.8*†	1.1*
RL seedl.	13	0	–	+	0.02	0.25	0.27	0.2	0.07	0.27
RL seedl.	3	3.7	–	+	1.0	7.0	8.0	0.0	1.0	1.0
SWO/RL[t]	4	1	+	+	0.7	2.8	3.5	0.5[h]	0.6[h]	1.1[h]

[z]Root rot severity based on a scale 0-4, with 0 = no root rot, 1-4 = increasingly severe root rot.
[y]Presence of *P. nicotianae* var. *parasitica* determined in growth medium by means of bio-assay (8).
[x]Presence of *F. solani* determined in the roots by isolating.
[w]Based on 10 cross sections per sample, * = significantly different from corresponding healthy plant according to Tukey's test (P = 0,05). † = significant difference between roots and stems for amorphous and filamentous occlusions respectively.
[v]Roots of ca. 4 mm diam. (On seedlings = primary root).
[u]Data for 4 mm diam. twigs.
[t]Sweet orange on rough lemon rootstock, 8 yr-old greenhouse reared.

parasitica was only recovered from the roots of one of these plants. Diseased plants showed extensive rotting of the feeder roots, and in two instances necrosis, of the primary root. No crown rot symptoms were observed.

Nature and incidence of xylem occlusions. Amorphous and filamentous occlusions were observed under the light microscope in the xylem of stems and primary roots of the sweet orange and rough lemon seedlings, as well as roots and twigs of the sweet orange trees on rough lemon rootstock (fig. 2). Both types of occlusions appeared similar in structure to those seen in blight-affected root samples. Filamentous occlusions in greenhouse grown plants were often lighter in colour (yellow to golden brown) compared to the dark brown filamentous occlusions in blight affected samples. Amorphous occlusions had the same structure and amber colour in both instances. In some plants, occlusions were concentrated in the center of roots and stems, while in others these were distributed more evenly through the xylem.

Gum-like deposits occurred between cells in the xylem of both sweet orange and rough lemon seedlings with root rot. These deposits were restricted to a narrow band in the young xylem. Under the light microscope they appeared golden yellow.

Scanning electron microscopy confirmed that amorphous occlusions in the greenhouse-grown plants were solid and similar in structure to those in blight-affected trees (Fig. 1). Amorphous occlusions which partially occluded vessels were also seen (Fig. 1). The majority of filamentous occlusions observed in healthy and diseased greenhouse grown plants were similar in structure to those present in blight-affected samples (Fig. 1). Some porous occlusions which exhibited amorphous and filamentous characteristics occurred in seedlings with *Phytophthora* root rot (Fig. 1). Filamentous occlusions with varying filament shapes and sizes were seen in diseased and healthy appearing greenhouse grown plants (Fig. 1).

Significantly more amorphous and filamentous occlusions were present in roots and stems of sweet orange and rough lemon seedlings with *Phytophthora* root rot than in healthy seedlings (Table 1).

Although more amophorus and filamentous occlusions occurred in the three rough lemon seedlings with *Fusarium*-associated root rot compared to healthy rough lemon seedlings (Table 1) this difference was not

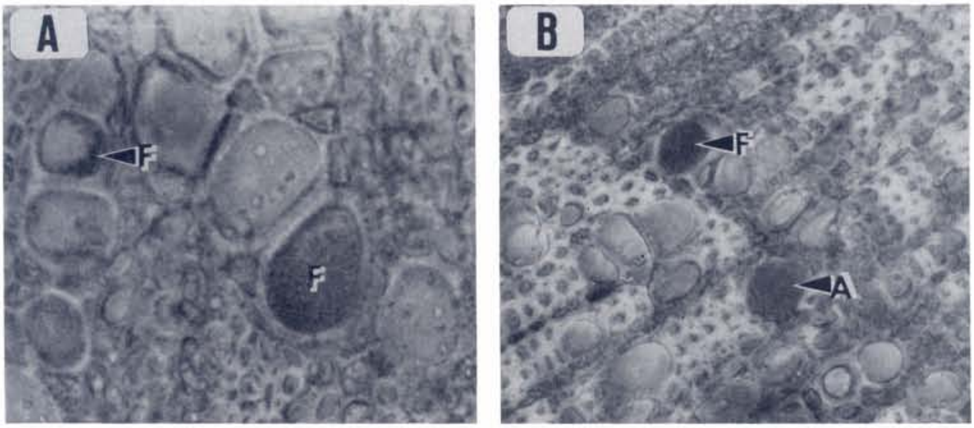

Fig. 2. Light microscopy of xylem occlusions in roots of citrus seedlings affected by *Phytophthora* root rot in the greenhouse. A) Golden-brown filamentous occlusions (F) completely and partially occluding xylem vessels in Valencia orange seedling (X550); B) Filamentous (F) and amber colored amorphous occlusion (A) in Valencia orange seedling (X150).

statistically significant, because of the small number of plants with only *Fusarium* associated root rot. When the number of amorphous occlusions in roots was compared versus the stems of diseased seedlings, rough lemon roots contained significantly more occlusions than stems while significant differences were not found in sweet orange seedlings. Significantly more filamentous occlusions occurred in the roots than in the stems of diseased plants.

DISCUSSION

The occurrence of high numbers of amorphous and fibrous occlusions in citrus plants affected by root rot and also in blight-affected trees (reported previously by Brlansky *et al.*, 1985) suggests that xylem occlusion in citrus is a stress reaction of the plant, which may be triggered by more than one pathological condition. Occlusion of plant tissue by gum-like deposits is a common plant pathological phenomenon (1). In accordance with these findings Nemec, Baker and Burnett (13) and Nemec, *et al.* (14) reported increased numbers of xylem occlusions in citrus plants affected by *Fusarium solani* and by dihydrofusarubin and isomarticin extracted from cultures of the fungus.

In accordance with previous findings (9, 10), symptomless roots of healthy-appearing citrus seedlings were found to be infected with *F. solani* in the present study. Few xylem occlusions occurred in these plants, whereas more occlusions of both types were present in three plants exhibiting *Fusarium* root rot (Table 1). These and previous findings (13, 14) seem to indicate that xylem occlusion is only stimulated by *F. solani* during the necrotrophic phase of parasitism. In contrast Graham *et al.* (7) observed no amorphous occlusions in trees affected by *Fusarium*-associated dry root rot. However, their study differed from ours in that they studied mature trees with a necrotic major root subtending the root crown whereas our seedlings were subjected to chronic feeder root decay over a period of at least 9 months. It is not known whether *Fusarium*-free plants are free of any occlusions.

In contrast with the high number of amorphous occlusions observed in plants with *Phytophthora* root rot in the present study, Brlansky *et al.* (4) found no amorphous occlusions in trees affected by root rot (crown rot) caused by the same fungus. This might indicate that amorphous occlusions are associated with root rather than stem disorders. It should be noted that plants affected by *Phytophthora* root rot in the present study were not free of *F. solani* (Table 1), apparently similar to any field-situation (9).

In accordance with the report by Brlansky *et al.* (4) we found that some occlusions which appear to be amorphous under the light microscope are not solid when viewed under the scanning electron microscope. It is therefore not clear whether the "resinous" occlusions observed by Nemec, *et al.* (13, 14) are of the solid amorphous type. The porous occlusions which appeared to have both amorphous and filamentous characteristics in the present study seem similar to those observed by Brlansky *et al.* (4) in concave gum affected trees. Furthermore, the occurrence of filamentous occlusions with apparently varying filament sizes and shapes agrees with the report by Graham *et al.* (7).

Although the citrus blight syndrome is characterized by increased numbers of amorphous occlusions in the xylem (4, 5) the present and previous studies (13, 14, 20) indicate that these occlusions are not strictly specific for blight, but may be used quantitatively, but not qualitatively as a diagnostic indicator of citrus blight, and only in addition to other diagnostic tests (18, 23). This is further supported by the fact that amorphous occlusions also occur in low numbers in apparently healthy trees (4, 6, 20).

Further experiments are planned to determine whether increased xylem occlusion is accompanied by zinc accumulation in the stems of citrus plants affected by root rot.

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