A Comparison of Histochemical Staining Reactions of the Xylem Occlusions in Trees Affected by Citrus Blight and Declinio

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ABSTRACT

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Declinio of citrus trees in Brazil, a vascular disorder of unknown cause, is similar to citrus blight in Florida. Both diseases are characterized by zinc accumulation in trunk wood and xylem plugging, which results in restricted water flow in diseased trees. This dysfunction is caused by the presence of xylem plugging. Amorphous and filamentous occlusions have been detected and reduced water flow has been correlated with amorphous plugs. The histochemical staining reactions of plugs found in xylem vessels in trees with declinio and blight were compared. With both diseases, the amorphous plugs reacted positively for callose, lignin, pectic substances, gums, proteins, and lipids. Filamentous plugs stained positively for lignins, gums, proteins, and lipids. These results showed that similar xylem plugs (amorphous and filamentous) were found in both declinio and blight, but the two plug types differ in their chemical composition.

Additional keywords: sweet orange, young tree decline

Declinio of citrus trees in Brazil and citrus blight in Florida are similar diseases of unknown cause characterized by wilt and dieback of the canopy (2,11,12). Declinio and blight are characterized by zinc accumulation in the trunk wood (13–15), reduced water uptake in the trunk and root xylem (2,4,8,11,12,16), and the presence of occlusions in the xylem vessels (1,3,4).

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Two types of xylem occlusions, filamentous and amorphous plugs, have been described in root, limb, and trunk xylem of declinio- and blight-affected trees. Filamentous plugs, consisting of a mass of fine fibers, are usually found in xylem vessels and walls. Amorphous plugs, consisting of solid material, are found in the lumen of xylem vessels and may completely block the vessel (2). Amorphous plugs have been associated with citrus blight and declinio and have not been found with other citrus declines (1,2).

The purpose of this study was to compare, by histochemical staining, the xylem occlusions found in trees affected by citrus blight in Florida and declinio in Brazil.

MATERIALS AND METHODS

Six blight-affected and six healthy sweet orange trees (*Citrus sinensis* (L.)

Osbeck) on rough lemon (*C. jambhiri* Lush) were selected in groves in central Florida, and six sweet orange trees on Rangpur lime (*C. limonia* Osbeck) showing declinio symptoms and six healthy trees were selected in groves in the northern part of Sao Paulo State, Brazil. All trees were at least 10–15 yr old.

A canopy rating of all sampled trees was made on a scale of 0 = healthy and 3 = severe decline, as previously described (7). Water uptake was determined by the syringe injection test (7) and zinc analysis was performed on the trunk wood (14) for diagnosis of blight and declinio as described previously (2).

Horizontal core samples 5–7 cm long and 5 mm in diameter were taken from the scion trunk wood above the bud union, with a Haglof increment borer. One core sample from each tree was taken directly above the hole used for the syringe injection test. After removal, the cores were stored in 0.066 M sodium phosphate buffer, pH 6.8 with 0.02% sodium azide. Cross sections, 20 μm thick, of the wood xylem, 2–3 cm from the cambium, were cut with a cryostat. The samples were viewed at ×100 by transmitted light microscopy for the presence of amorphous and filamentous occlusions, as described previously (2). Sections from blight- and declinio-affected trees were prepared for scanning electron microscopy (SEM), as previously described (1), and were viewed to verify the similarity of the occlusions in blight- and declinio-affected trees.

Histochemical tests, as previously described (5,6,10), were made on 10–12 freshly cut sections of trunk wood taken from each tree affected by blight and declinio. The following stains were applied: 1) iodide-sulfuric acid to detect cellulose; 2) zinc-chloro iodide to detect cellulose; 3) aniline blue (visible light) to detect callose; 4) aniline blue (ultraviolet light) to detect callose; 5) IKI reactions to detect starch; 6) phloroglucinol to detect lignin; 7) Maule stain to detect lignin; 8) potassium permanganate to detect lignin; 9) ferric chloride to detect phenolic compounds and tannins; 10) ruthenium red to detect pectic substances; 11) orcinol to detect gums; 12) Ninhydrin-Schiff's reagent to detect proteins; 13) Sudan IV to detect neutral lipids; 14) Nile blue to detect total lipids; and 15) Sudan black B to detect lipids. After treatment, the plus or minus colorimetric reactions of the amorphous and filamentous plugs were observed at ×100 with a light microscope.

RESULTS AND DISCUSSION

The histochemical tests with different stains indicated differences in the composition of amorphous and filamentous plugs. However, the composition of either amorphous or filamentous plugs was the same with declinio and blight because no staining differences of the same plug were detected between the two diseases. The amorphous plugs with both blight and declinio gave positive reactions for callose, lignin, pectic substances, gums, protein, neutral lipids by Sudan IV, and total lipids by the Nile blue and Sudan black B tests (Table 1). The filamentous plugs stained positively for lignin, gums, protein, neutral lipids (Sudan IV), and total lipids (Sudan black B).

No cellulose, starch, phenolic compounds, or tannins were detected in the amorphous plugs with these tests. There was no cellulose, callose, starch, pectic substances, phenolic compounds, or tannins found in the filamentous plugs. A negative staining reaction with Nile blue was obtained for the filamentous plugs. Nile blue is used for total lipids and stains neutral lipids red, whereas free fatty acids and phospholipids stain blue. Because positive results were obtained with both Sudan IV for neutral lipids and Sudan black B for fatty acids and phospholipids, it would have been expected that the filamentous plugs would stain positive with Nile blue. The reason for this staining reaction is not known. Further staining with Orange G-aniline blue for detection of phospholipids (6) also failed to stain the filamentous plugs. Similar results were obtained for both declinio and blight. Nemec and Kopp (9) showed the presence of lipids, using Sudan black B stain, in the occlusions of blighted trees. We obtained similar results.

The similarity of both types of xylem occlusions in blight- and declinio-affected trees was verified using SEM (Fig. 1). The amorphous plugs in both blight- and declinio-affected trees were solid and nonporous in structure (Fig. 1A–D), while the filamentous plugs consisted of a threadlike material on both types of affected trees (Fig. 1E–H). The filamentous plugs at times often appeared coated with some type of material (Fig. 1F). These results agreed with those found previously on the structural nature of these plugs.

Brlansky et al (1), using light and electron microscopy, showed that blight amorphous plugs differ structurally from blight filamentous plugs. The amorphous occlusions are dense and nonporous, filling almost the entire vessel lumen, while the filamentous occlusions consisted

Table 1. Histochemical tests on amorphous and filamentous plugs in blight- and declinio-affected trees

Compound detected	Histochemical stain	Blight[a]		Declinio	
		Amorphous plugs	Filamentous plugs	Amorphous plugs	Filamentous plugs
Cellulose	Iodide sulfuric acid	–[b]	–	–	–
Cellulose	Zinc chloro iodide	–	–	–	–
Callose	Aniline blue (visible light)	+	–	+	–
Callose	Aniline blue (UV light)	+	–	+	–
Starch	IKI reactions	–	–	–	–
Lignin	Phloroglucinol	+	+	+	+
Lignin	Maule stain	+	+	+	+
Lignin	Potassium permanganate	+	+	+	+
Phenolic compounds and tannins	Ferric chloride	–	–	–	–
Pectic substances	Ruthenium red	+	–	+	–
Gums	Orcinol	+	+	+	+
Proteins	Ninhydrin-Schiff's reagent	+	+	+	+
Neutral lipids	Sudan IV	+	+	+	+
Total lipids	Nile blue	+	–[c]	+	–[c]
Acidic lipids	Sudan black B	+	+	+	+

[a] Tests were based on one horizontal core sampled from each of six blight-affected sweet orange trees (*Citrus sinensis*) on rough lemon rootstock (*C. jambhiri*) and six declinio-affected sweet orange trees on rangpur lime (*C. limonia*) rootstock. Ten to 12 cryostat sections were used from each core sample for staining.

[b] + = Positive color reaction; – = no color reaction.

[c] No red staining for neutral lipids or blue staining for free fatty acids and phospholipids was observed.

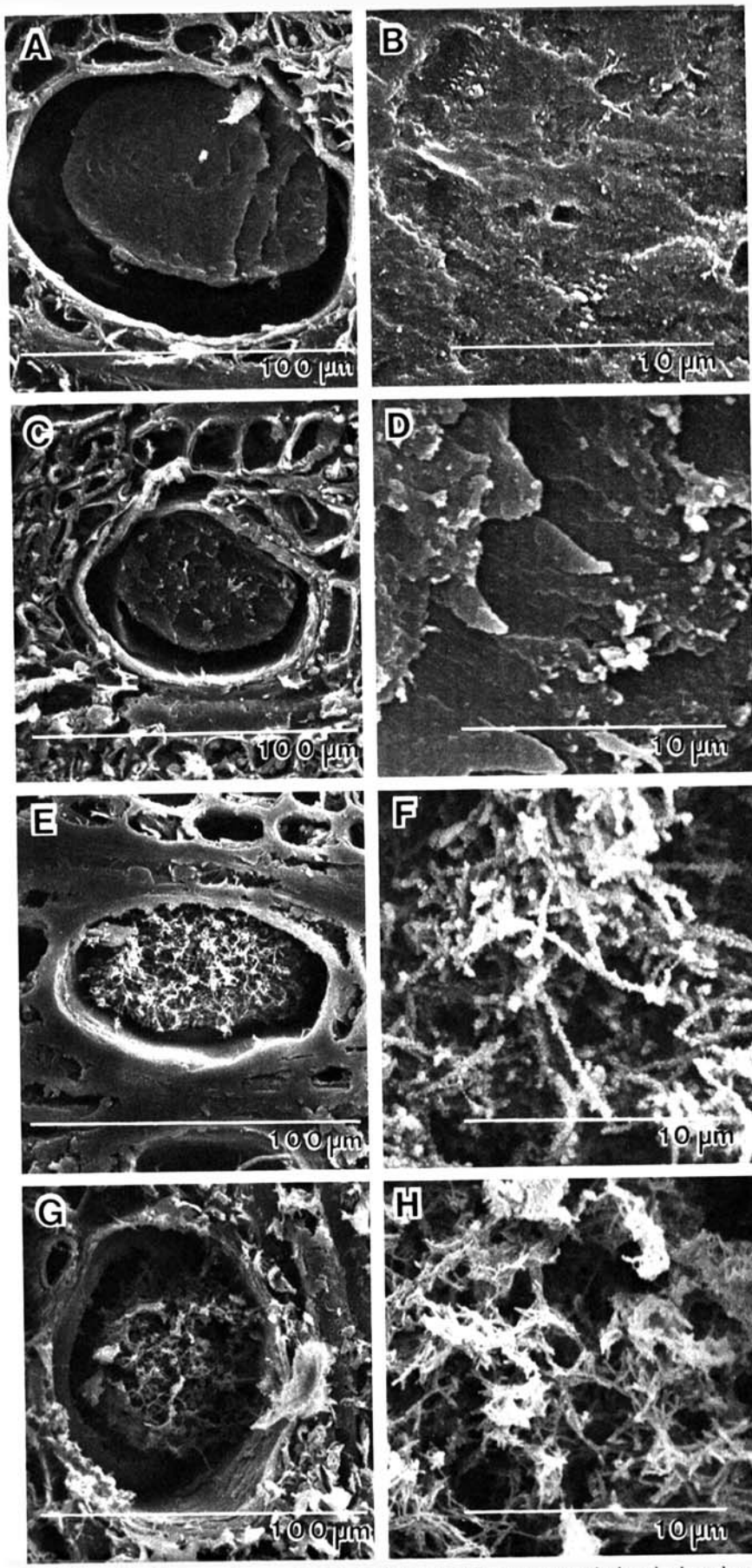

Fig. 1. Scanning electron micrographs of amorphous and filamentous occlusions in the xylem of trees affected with blight and declinio. **(A,B)** Amorphous occlusion in blight-affected tree (×500 and ×4,000), **(C,D)** amorphous occlusion in declinio-affected tree (×500 and ×4,000), **(E,F)** filamentous plug in blight-affected tree (×500 and ×4,000), and **(G,H)** filamentous plug in declinio-affected tree (×500 and ×4,000).

of strands of threadlike material in the vessel lumens and along the vessel walls. The filamentous plugs are present in healthy as well as blight- and declinio-affected trees, while the amorphous plugs are characteristic of blight and declinio (2).

From this work, it appears that declinio and blight are similar, and the amorphous plugs are different in composition from the filamentous plugs. More chemical studies are needed to further define the composition of these xylem occlusions.

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