Relationship of Xylem Plugging to Reduced Water Uptake and Symptom Development in Citrus Trees with Blight and Blightlike Declines

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ABSTRACT

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Citrus trees with declines of unknown etiology—which are called blight in Florida, "declinio" in Brazil, and "declinamiento" in Argentina—all had significantly higher zinc levels in the trunk wood, lower water uptakes, and higher frequency of amorphous occlusions in the xylem than did comparable healthy trees. Filamentous plugs were not consistently associated with declining trees, and the number of filamentous plugs was not highly correlated with water uptake ($r = -0.43$). The logarithm of the number of amorphous plugs was significantly correlated with water uptake ($r = -0.61$). The canopy rating was significantly correlated with the logarithm of the number of amorphous occlusions ($r = 0.71$). When 5–10 amorphous plugs per 200 xylem vessels were present, water uptake was reduced to 0.30 ml/sec as compared to 0.82 ml/sec in healthy trees, and decline symptoms developed.

Additional key words: *Citrus sinensis*, fruta bolita, marchitamiento repentino, sandhill decline, young tree decline.

Citrus blight, a decline disease of unknown etiology, has been characterized by reduced water conductivity of the xylem of trunks, limbs, and roots (5,16) and high levels of zinc in the trunk wood (15). Two types of xylem blockage structures, filamentous and amorphous occlusions, have been described in the xylem of the roots, limbs, and trunk (1,4,6,10). Filamentous plugs were described by Childs (3) as the primary blockage in the root xylem of blight-affected trees, and he indicated this to be diagnostic of blight. Nemec et al (10) reported that gum (amorphous) plugs were the main resistance along the cambium in the trunk of blight-affected trees, but also listed filamentous plugs and narrow vessels as contributing to reduced water movement. Vandermolen et al (14) stated that amorphous plugging was more responsible for the blight symptoms than was filamentous plugging. Nemec (9) and Vandermolen (13) later reported detailed studies on the filamentous type plugging associated with citrus blight. The natures and locations of these two types of plugs were studied by Cohen et al (6), and an association was made between the reduced water uptake in the trunk and major scaffold limbs of blighted trees and the presence of amorphous plugs. Filamentous plugging occurred in both healthy and decline trees and was most common near the pith at the center of the wood. Water conductivity was the lowest where the largest number of plugs was found, and amorphous plugs were the most prevalent type.

Although canopy decline in blight-affected citrus trees is presumed to be due in part to restricted water flow caused by xylem plugging, no relationship between these factors has been established. Lee et al (8) developed a new technique which allows rapid determination of the water uptake in localized areas of trunks of healthy and suspected blight-affected citrus trees. This technique uses a syringe to forcefully inject water into the trunk xylem where plugging may occur.

The purpose of this study was to determine quantitatively the relationship between reduced water uptake, the number and type of xylem occlusions, and development of symptoms in citrus trees with blight and blight-like declines. Trees on various rootstocks were studied in Florida, Argentina, and Brazil to determine if blight, declinamiento, and declinio have similar characteristics.

MATERIALS AND METHODS

Declining and healthy sweet orange (*Citrus sinensis* (L.) Osb.) on various rootstocks (Table 1) were selected in groves in central Florida, in the states of São Paulo and Bahia, Brazil, and in the provinces of Misiones, Entre Rios, and Corrientes, Argentina. Zinc analysis of the scion trunk wood was conducted by the method of Wutscher et al (15), and water uptake was determined by the syringe injection test (8). Canopy ratings of all trees sampled were made on the scale of 0 = healthy to 3 = severe decline as previously described (7).

Horizontal core samples 5–7 cm long were taken from the scion trunk wood with a 5-mm Haglof increment borer (Forestry Suppliers, Jackson, MS 39204). The core was taken directly above the hole drilled for the syringe water injection test. Cores were fixed immediately in 3% glutaraldehyde in 0.066 M sodium-potassium phosphate buffer, pH 6.8, for 8 hr or overnight. Then the glutaraldehyde solution was removed and replaced with phosphate buffer and the cores were washed for at least 1 hr. The portion of the core 2–3 cm from the cambium (corresponding to the site directly above the water injection area) was cut from the core. Transverse sections 30–40 μm thick were prepared from the center one-third of this portion of the core by using an AO Spencer model 860 sliding microtome. The remaining portions were prepared for scanning

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electron microscopy (SEM) as previously described (6). Transverse sections were mounted on microscope slides, and the number of filamentous and amorphous plugs was counted on the microscope in 200 xylem vessels in random microscope fields at ×100.

Mean values for canopy ratings, zinc content of trunk wood, water uptake, and the number of plugs per 200 vessels were compared for declining and healthy trees in each location by using Student's *t*-test. The number of filamentous, amorphous, and total plugs per 200 vessels was plotted versus water uptake and the curve best fitting the data was determined by regression analyses. Similarly, water uptake and numbers of amorphous plugs were plotted versus canopy ratings to determine the relationship between these parameters. Correlation coefficients were calculated for the relationship between each factor.

RESULTS

Filamentous and amorphous plugs in declinio- and declinamiento-affected trees were identical by both light microscopy and SEM, to those previously described for blight-affected trees (6). Filamentous plugs were brown and occurred most frequently at vessel end walls. Amorphous plugs were yellow to light gold, solid in appearance, and often occluded the length and width of a xylem vessel.

The means of the canopy ratings, zinc content of the trunk wood, water uptake, and the number of vessel plugs (filamentous, amorphous, and total) are summarized in Table 1. Zinc levels were significantly higher in decline trees than in healthy trees in all cases. Water uptake rates were significantly lower in diseased trees than in healthy trees in all locations and rootstocks tested. There were significant differences in the number of filamentous plugs between declining and healthy trees only in trees on trifoliate rootstock in Florida and in Misiones, Argentina. In all comparisons except one, there were significantly more amorphous plugs in declining than in healthy trees. The total number of vessel plugs was significantly higher in declining than in healthy trees in all instances except in samples from Corrientes, Argentina. However, in this area there was a substantial difference in the numbers of plugs in decline versus healthy trees (Table 1).

When water uptake data from all locations were plotted against the canopy rating, the data best fit a negative logarithmic function ($r = -0.86$) (Fig. 1). On the average, trees rated as healthy took up water at 0.82 ml/sec. At the time when visual symptoms first became apparent and blight was distinguishable from the normal variation in canopy condition due to other factors (visual rating, 0.5), water uptake was 0.30 ml/sec. Once visual symptoms appeared, water uptake declined very rapidly to near zero.

When water uptake was plotted against the number of amorphous, filamentous, and total plugs in the xylem vessels, negative logarithmic curves provided the best fit for the data (Fig. 2). Separate regression analyses and curve fitting for each location

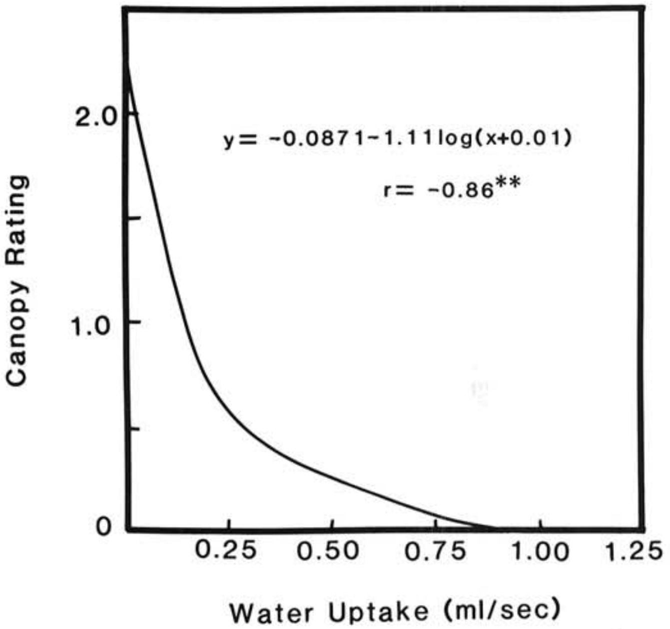

Fig. 1. Relationship between canopy symptoms and water uptake by the syringe injection technique into the trunk of healthy and declining citrus trees. Canopy condition rated on a scale of: 0 = healthy, 1 = mild decline, 2 = moderate decline, and 3 = severe decline. Correlation significant at $P = 0.01$ (**).

TABLE 1. Characteristics of healthy and declining sweet orange trees in Florida, Brazil, and Argentina

Location	Rootstock[a]	Trees sampled (no.)	Canopy rating[b]	Zinc (μg/g)	Water uptake (ml/sec)	Plugs per 200 vessels		
						Filamentous	Amorphous	Total
Florida	RL	8	0.0	2.60	0.92	2.9	0.9	3.8
		8	1.8	8.50*[c]	0.00*	6.1 ns	25.5*	31.6*
Florida	T	6	0.0	2.40	0.62	0.5	0.2	0.7
		6	2.0	6.70*	0.00*	22.8*	38.8*	61.6*
Brazil (São Paulo)	R	6	0.0	4.30	0.56	1.3	2.5	3.8
		6	2.0	8.60*	0.00*	3.5 ns	25.5*	29.0*
Brazil (Bahia)	R	5	0.0	7.90	0.38	5.3	2.8	8.1
		5	2.0	30.70*	0.02*	11.5 ns	45.5*	57.0*
Argentina (Entre Rios)	T	11	0.0	1.82	0.85	2.6	0.4	3.0
		11	1.9	3.30*	0.04*	7.7 ns	10.2*	17.9*
Argentina (Entre Rios)	RL	5	0.0	2.30	1.24	1.4	0.4	1.6
		5	1.3	5.65*	0.03*	4.6 ns	8.2 ns	12.8*
Argentina (Corrientes)	T	9	0.0	0.94	0.75	1.1	0.6	1.7
		9	1.1	4.06*	0.10*	6.9 ns	8.9*	15.8 ns
Argentina (Misiones)	T	16	0.0	1.52	1.06	3.9	0.1	4.0
		16	2.1	3.73*	0.08*	10.7*	9.5*	20.2*

[a] RL = Rough lemon (*Citrus jambhiri* Lush); R = Rangpur lime (*C. limonia* Osb.); and T = Trifoliate orange (*Poncirus trifoliata* (L.) Raf.).

[b] Canopy condition rated on a scale of: 0 = healthy, 1 = mild decline, 2 = moderate decline, and 3 = severe decline.

[c] Asterisk indicates means significantly different from the corresponding healthy control according to the Student's *t*-test at $P = 0.05$; ns = not significant.

indicated that the relationship between plugging and water uptake was similar for all sites (Table 2). As the number of amorphous plugs increased, water uptake decreased rapidly (Fig. 2a). At 0.30 ml/sec, which was the water uptake level for incipient blighted trees (Fig. 1), there was an average of eight amorphous plugs per 200 vessels. Moderately to severely blighted trees that took up no water averaged about 40 amorphous plugs per 200 vessels.

Over all locations, there was also a significant correlation of the number of filamentous plugs and the total number of plugs with water uptake. However, the correlation coefficient for filamentous plugs was lower than for amorphous plugs (Fig. 2b). Also, water uptake was not significantly correlated with numbers of filamentous plugs in all locations, whereas the correlation with numbers of amorphous plugs was significant at all sites (Table 2). When the number of filamentous plugs was added to the number of amorphous plugs, the correlation was no better than with the amorphous plugs alone. Furthermore, utilizing step-wise multiple regression analysis, the logarithm of the number of filamentous plugs was a significant factor in the equation, but it accounted for less than one-fourth of the variability explained by the logarithm of the amorphous plugs. There was a linear correlation coefficient of +0.37 between the number of amorphous and the number of filamentous plugs, indicating that the correlation of filamentous plugs with water uptake is partially due to their association with amorphous plugs.

The canopy rating was significantly related to the $\log_{10}$ of the number of amorphous plugs ($r = 0.71$) (Fig. 3). Less than 10 amorphous plugs per 200 vessels brought about sufficient water inhibition to cause mild blight symptoms. Blight severity increased further as the number of amorphous plugs increased.

DISCUSSION

In this study, the citrus tree declines known as blight in Florida, declinamiento in Argentina, and declinio in Brazil had similar characteristics. Zinc levels in the trunk wood were significantly higher in trees affected by all three declines, water uptake was significantly reduced, and the number of amorphous plugs in the xylem was higher. Although they occur in geographically different areas and are found on different rootstocks, all three declines are probably the same or are very closely related.

Zinc levels in the decline trees were higher than in healthy trees from the same location and provided a useful diagnostic tool. However, since zinc levels in decline trees in Argentina were lower than levels in healthy trees in Brazil, it appears unlikely that zinc accumulation alone causes the symptoms.

Amorphous plugs were most prevalent in declining trees, and significant differences from healthy trees occurred in all locations. Cohen et al (6) consistently associated amorphous plugs in blighted trees in Florida with reduced water flow, whereas much of the previous work on blight had focused on the presence of the filamentous plugs (2–4,9,13). In most cases, filamentous plugging was not significantly higher in decline than in healthy trees (Table 1). Filamentous plugging was not as well correlated with the reduction in water uptake as was amorphous plugging. Addition of the number of filamentous plugs to the amorphous plugs did not increase the percentage of the variability accounted for in the water uptake. Moreover, we have found filamentous plugs in other decline diseases which are not associated with reduced water uptake (*unpublished*).

Young (16) and Cohen et al (6) demonstrated that reduced water transport in blight-affected trees is due primarily to plugging in the

TABLE 2. Regression analysis of water uptake (ml/sec) versus the logarithm of the number of filamentous, amorphous, and total plugs per 200 xylem vessels

			Correlation coefficients (r)		
Location	Rootstock[a]	N[b]	Filamentous	Amorphous	Total
Argentina (Entre Rios)	T	24	−0.42*[c]	−0.59**	−0.56**
Argentina (Corrientes)	T	18	−0.40	−0.64**	−0.61**
Argentina (Misiones)	T	23	−0.67**	−0.69**	−0.74**
Florida	T	12	−0.81**	−0.81**	−0.83**
Brazil (São Paulo - Bahia)	R	30	−0.38*	−0.52**	−0.48**
Florida	RL	21	−0.10	−0.57**	−0.51**
Overall		128	−0.43**	−0.61**	−0.60**
R^2			18.3	37.7	36.2

[a] T = trifoliate orange (*Poncirus trifoliata* (L.) Raf.); R = Rangpur lime (*C. limonia* Osb.); and RL = Rough lemon (*C. jambhiri* Lush).

[b] Number of trees tested.

[c] Correlation coefficients significant at $P = 0.05$ (*) or $P = 0.01$ (**).

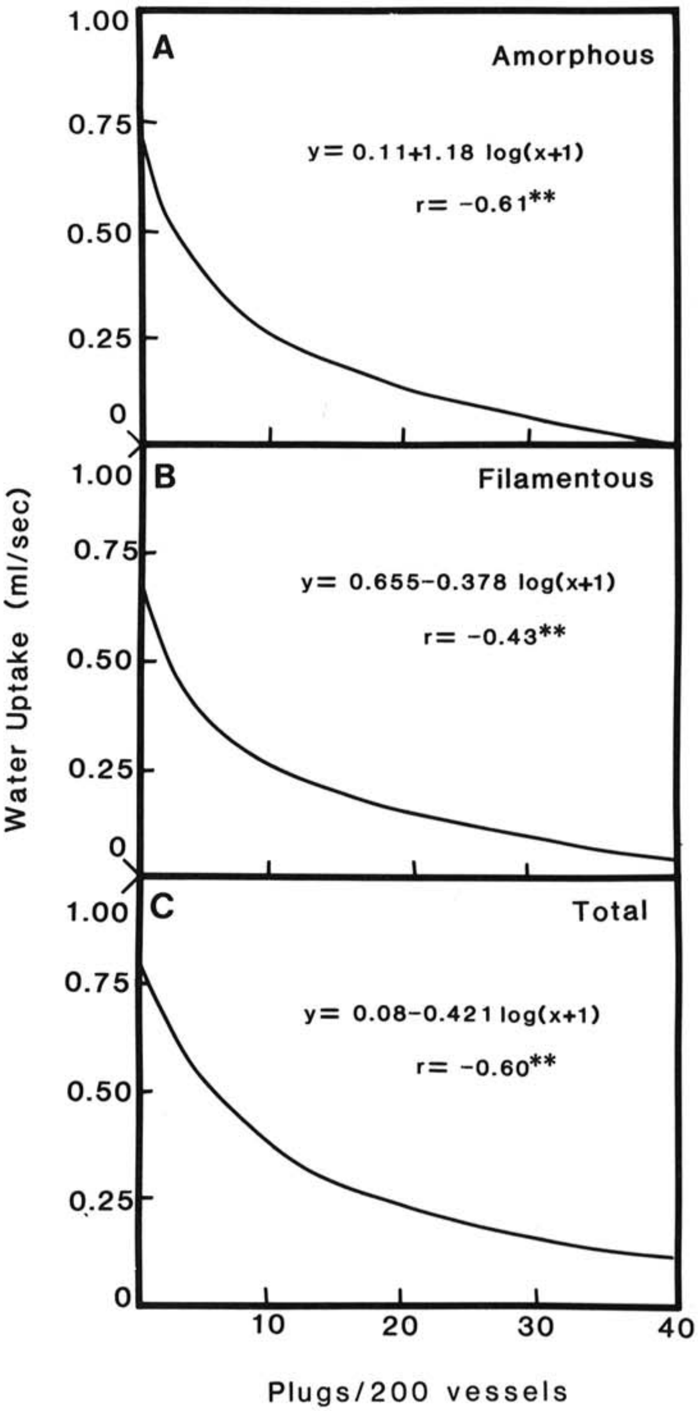

Fig. 2. Relationship between water uptake by the syringe injection method into the trunk to the number of xylem plugs in healthy and declining citrus trees. **A,** Amorphous plugs; **B,** filamentous plugs; **C,** total plugs. Correlations significant at $P = 0.01$ (**).

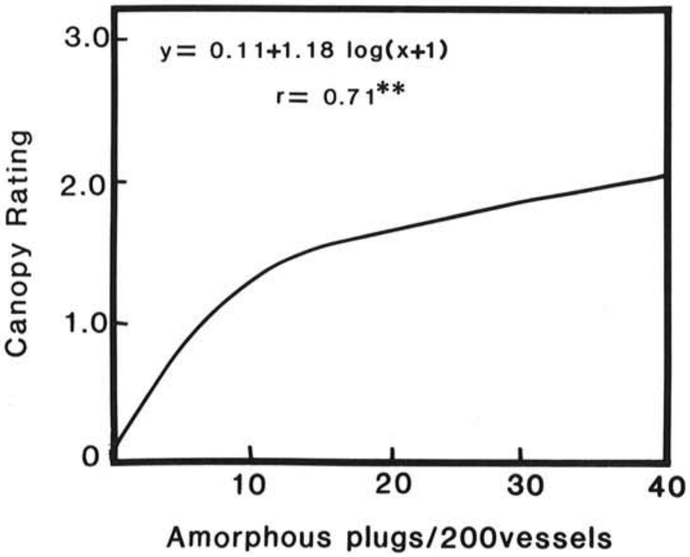

Fig. 3. Relationship of canopy rating to the number of amorphous plugs. Canopy condition rated on a scale of: 0 = healthy, 1 = mild decline, 2 = moderate decline, and 3 = severe decline. Correlation significant at $P = 0.01$ (**).

trunk and scaffold limbs, and to a lesser extent in smaller limbs and roots. Water transport is impeded in some, but not all of the smaller roots on blight-affected trees. Syvertsen et al (11) found that blight did not affect diurnal leaf water potentials and leaf stomatal conductances and concluded that water stress symptoms were related to increased resistance to water transport. Likewise, the hydraulic conductivity of feeder roots was unaffected by the disease (12). We have not found a tree with blight canopy symptoms, zinc accumulation, and reduced water uptake that does not have amorphous occlusions (*unpublished*).

Therefore, we suspect that the amorphous plugs are responsible for lower water uptake and that reduced water transport in the trunk brings about the canopy decline in citrus blight and associated declines. Current studies on the ontogeny of amorphous plugging in presymptomatic trees may determine whether there is a cause and effect relationship between plugging and canopy symptoms. Filamentous plugs may contribute to restricted water flow but do not appear to be specific for citrus blight.

In this study, we have shown a relationship between water uptake, the number of amorphous plugs, and the decline of the tree canopy. Lee et al (8) estimated that at a threshold of water uptake of <0.3 ml/sec citrus trees should be considered as being affected by citrus blight. In the present study, water uptake of 0.3 ml/sec corresponded to a canopy rating of 0.5 at which stage of disease development 5–10 amorphous plugs in 200 vessels were present. Water uptake is substantially reduced even when relatively few amorphous plugs are present in a given transverse section of the xylem. With the techniques used in this study, we probably examined only a small percentage of the vessels that are actually blocked in the volume of xylem in which the water uptake is reduced. Five to 10 amorphous plugs per 200 xylem vessels in a short length of xylem probably represents a much higher percentage of the vessels blocked if the total length of each vessel is considered. Since amorphous plugging lines or coats vessel walls in addition to the actual volume covered by the plug, a reduction in lateral water movement probably also occurs.

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