Title

Effect of Amorphous Plugs, Filamentous Plugs, and Depth into Trunk Xylem on the Water Flow in Declining and Healthy Citrus Trees

Permalink

https://escholarship.org/uc/item/8tz9b41d

Journal

International Organization of Citrus Virologists Conference Proceedings (1957-2010), 10(10)

ISSN

2313-5123

Authors

Timmer, Lavern W.
Brlansky, R. H.
Graham, James H.
et al.

Publication Date

1988

DOI

10.5070/C58tz9b41d

Peer reviewed

Effect of Amorphous Plugs, Filamentous Plugs, and Depth into Trunk Xylem on the Water Flow in Declining and Healthy Citrus Trees[z]

L. W. Timmer, R. H. Brlansky, J. H. Graham, J. P. Agostini, and R. F. Lee

ABSTRACT. Water flow in the xylem of trees affected by citrus blight and other blightlike declines is restricted by xylem occlusions. Cores were collected from trunks of trees affected by blight in Florida and declinamiento in Argentina, from apparently healthy, low vigor trees in Argentina, from trees affected by Phytophthora foot rot and tristeza decline in Florida, and from healthy trees in Florida and Argentina. Water flow and the number of amorphous and filamentous plugs were determined in the 0 to 1, 1 to 2, 2 to 3, and 3 to 4 cm segments of the cores measuring from the cambium. There were significant negative linear correlations between water flow and the log of the numbers of amorphous ($r = -0.55$) and filamentous ($r = -0.48$) plugs and between the depth of the sample ($r = -0.39$) and water flow. In stepwise multiple linear regression, the log of the numbers of amorphous plugs accounted for 26% of the variability in water flow and depth for 12%; whereas, the log of the number of filamentous plugs accounted for only 2% of the variability. The model—water flow (ml/sec) = 0.55-0.135 log of the number of amorphous plugs -0.07 depth (cm) -0.07 log of the number of filamentous plugs accounted for 40% of the variability in water flow. Thus, amorphous and filamentous plugs and depth contribute to the reduction in water flow, but amorphous plugs are the most important factor reducing water flow in blighted citrus trees.
Index words. xylem occlusions, blight, declinamiento.

Cohen (5) first demonstrated that trees affected by citrus blight, a disease of unknown etiology, had reduced uptake of water into the trunk. Young and Garnsey (15) found that water movement was greatly restricted in the trunks of citrus trees with blight, less so in large limbs and roots, and only occasionally affected in small limbs and roots. Filamentous plugs were described in roots of blighted trees by Childs and Carlysle (4), who considered them to be the primary blockage structures and to be diagnostic of the disease. Nemec *et al.* (10) reported that gum (amorphous) plugs were the main resistance to water flow in the trunk, but also noted that filamentous plugs and narrow vessels could contribute to restricted water movement. VanderMolen *et al.* (13) indicated that amorphous plugs were more responsible for blight symptoms than were filamentous plugs. Cohen *et al.* (6) found that reduced water flow was more closely associated with amorphous than with filamentous plugs. Brlansky *et al.* (3) and Graham *et al.* (7) found filamentous plugs in trees affected by many decline diseases but found true amorphous plugs only in trees affected by blight and similar decline diseases. The morphology of filamentous and amorphous plugs have been described in detail (3, 10, 12), but their chemical nature is complex and poorly defined (10, 13).

Since the development of the syringe injection technique (8), it has been possible to more precisely measure water uptake into tree trunks. Thus, it was possible to demonstrate that as water uptake by this technique decreases below 0.2 to 0.3 ml/sec, the tree begins to decline (2). As amorphous plugs in cross sections of

[z]Florida Agricultural Experiment Station Journal Series No. 7920.

vessels increase to 8 to 10 amorphous plugs/200 vessels, water uptake is restricted (2). In regression analyses, the numbers of amorphous plugs accounted for 36% of the variation in water uptake and filamentous plugs accounted for 18%. Adding amorphous and filamentous plugs together, however, did not account for more of the variation than the amorphous plugs alone. Because the two plug types occur together in blighted citrus trees, it is difficult to separate the effects of each on water uptake.

A device developed by Albrigo *et al.* (1) allows measurement of water flow through the segments of cores in which plug counts are actually made. The purpose of this paper was to examine more critically the contribution of amorphous and filamentous plugs to the reduction in water flow in blighted citrus using this technique.

MATERIALS AND METHODS

To determine the effect of plug type and depth into trunk xylem on water flow, cores were collected from all of the trees listed in table 1.

Wood samples for zinc analysis were collected, dried, and analyzed by atomic absorption spectroscopy as described by Wutscher *et al.* (14).

The syringe injection method (8) was used to measure water uptake into the trunk. Horizontal core samples, about 5 cm long, were taken from the scion trunk wood about 20 to 30 cm above the budunion with a 5 mm Haglof increment borer (Forestry Suppliers, Jackson, MS 39204) directly above the hole drilled for the syringe water injection test. Cores were fixed overnight in 3% glutaraldehyde in 0.066 M Na-K phosphate buffer, pH 6.8. The glutaraldehyde solution was removed and the cores were stored in the phosphate buffer at 4 C.

Water flow through the cores was determined in four 1-cm segments measured from the cambium using a device described by Albrigo *et al.* (1). The apparatus consisted of metal tubing with an inside diameter of 7 mm with the ends of the tubes cut so that they could be clamped snugly around the curvature of the top and the bottom of the core. The 7 mm diameter opening was centered over the desired segment, the core oriented in the direction of normal water flow, and a vacuum of 625 ± 35 mm Hg was applied. Water was drawn through the core for 30 sec and the average flow in ml/sec calculated.

After water flow determinations, transverse sections 30 to 40 μm thick were cut from the center one-third of each segment using an AO Spencer Model 860 sliding microtome. The number of filamentous and amorphous plugs in 200 vessels were counted in random microscope fields at 100X.

Simple regression analyses were used to determine the effect of filamentous and amorphous plugs from all core segments on water flow, the effect of depth from the cambium to the center of the trunk of water flow, and the relationship between the number of amorphous and filamentous plugs. Subsequently, stepwise multiple linear regression analyses were used to determine the contribution each factor made to the reduction in water flow.

RESULTS AND DISCUSSION

In order to achieve a wide range of water flow, from 0 to 1.0 ml/sec, cores from blighted, healthy, and low vigor trees were used as well as a few from trees affected by Phytophthora root rot and tristeza decline. Trees from Misiones, Argentina, which have been designated "low vigor" trees (11), were particularly advantageous for this type of analysis since they had both plug types but usually had some water uptake (table 1). Thus, the majority of trees used in the study were of this type. If blighted trees alone were analyzed, water flow was near zero in all segments except the outermost; and it was impossible to distinguish the effects of amorphous and filamentous plugs on

TABLE 1
CHARACTERISTICS OF TREES SAMPLED TO DETERMINE THE RELATIONSHIP OF XYLEM PLUGGING AND WATER FLOW IN XYLEM CORE SAMPLES

Tree condition	Scion[z]	Rootstock[z]	Year sampled	Location	No. of tree	Water uptake (ml/sec)[y]	Zinc (µg/g)[x]	Avg. no. of plugs/200 vessels	
								Amorphous	Filamentous
Healthy	Val	RL	1984	Auburndale, FL	6	0.68	1.7	0.3	1.8
	Val	trif	1984	Montecarlo, Arg.	6	0.98	1.7	0.2	0.3
	Ham	trif	1984	Ft Pierce, FL	6	0.81	4.8	1.5	4.6
	Val	RL	1986	Barnum City, FL	10	0.70	—	0.4	1.3
Blight	Val	RL	1984	Auburndale, FL	8	0.01	16.0	20.3	4.3
	Ham	trif	1984	Ft. Pierce, FL	6	0.00	9.4	21.9	10.8
	Val	trif	1984	Eldorado, Arg.	6	0.00	4.1	20.2	11.2
Low vigor[w]	Val	sweet	1984	Eldorado, Arg.	5	0.22	8.9	22.1	10.6
	Val	cleo	1984	Eldorado, Arg.	6	0.07	—	11.9	15.0
	Val	Rang	1984	Eldorado, Arg.	6	0.11	—	22.4	33.8
	Val	RL	1984	Eldorado, Arg.	4	0.05	10.7	11.8	5.9
	Val	sweet	1986	Eldorado, Arg.	6	0.40	—	28.8	23.3
	Val	cleo	1986	Eldorado, Arg.	6	0.10	—	18.4	26.8
	Val	Rang	1986	Eldorado, Arg.	6	0.07	—	17.4	36.6
	Val	RL	1986	Eldorado, Arg.	6	0.11	—	16.1	8.0
Root rot[v]	Val	sweet	1986	Highland City, FL	9	—	—	0.6	3.0
Tristeza decline	Val	sour	1986	Ft. Pierce, FL	6	0.61	—	1.3	1.8

[z]Val = Valencia sweet orange, RL = rough lemon, trif = trifoliate orange, Ham = Hamlin sweet orange, sweet = sweet orange, cleo = cleopatra mandarin, Rang = Rangpur lime.
[y]Water uptake measured by the syringe injection test.
[x]Zinc content of trunk wood.
[w]Trees with low vigor, but not declined, which have low water uptake.
[v]Trees with root rot caused by *Phytophthora* sp.

water flow. However, some blighted trees were included in order to have sections with zero water flow. Healthy trees were used in order to have sections with high water flow with no plugging. The trees affected with Phytophthora root rot and tristeza decline had little plugging and, for purposes of this study, could be considered with the healthy trees (table 1). Attempts to find trees with only filamentous or amorphous plugs in order to determine more directly the effect of each plug type on water flow were unsuccessful.

In simple linear regression analyses of water flow, there was a significant negative correlation between water flow and the numbers of amorphous, filamentous, or total plugs. As in previous studies (2, 11), adding the two plug types together did not appreciably improve the correlation coefficient (table 2). Since the relationship of water flow and plugging appeared to fit a negative logarithmic function more closely than a linear function, regression analyses were carried out with the log of the number of plugs. This improved the correlation coefficients (table 2, fig. 1A, B) but did not affect the comparisons of the effects of the plug types. There was also a positive correlation between the number of amorphous and filamentous plugs which confounds interpretation of the effects of amorphous, filamentous, and total plugs on water flow. The depth of the sample, i.e., 1, 2, 3, or 4 cm deep in the cores, also had a significant effect on water flow when all trees were considered together (table 2). Since there was a significant negative correlation between water flow and depth in healthy trees (fig. 2), depth alone is a factor independent of the increase of plugging with depth.

TABLE 2
SIMPLE CORRELATION COEFFICIENTS BETWEEN WATER FLOW, NUMBER OF AMORPHOUS AND FILAMENTOUS PLUGS, AND DEPTH INTO TRUNK OF XYLEM CORE SAMPLES

Factors	r value[z]
Water flow vs. amorphous	–0.41
Water flow vs. filamentous	–0.38
Water flow vs. total plugs	–0.48
Water flow vs. log amorphous	–0.55
Water flow vs. log filamentous	–0.48
Water flow vs. log total plugs	–0.55
Amorphous vs. filamentous	+0.35
Water flow vs. depth (healthy)	–0.44
Water flow vs. depth (all)	–0.39

[z]All r values are significant at $P \leq 0.01$.

It would appear from this type of analysis that filamentous plugs might contribute substantially to reduced water flow. However, as observed previously, the correlation of water flow to total plugs is not really improved over those considering each plug type separately.

The contribution of each factor to reduction in water flow was more clearly established by stepwise multiple linear regression of the $\log_{10}$ of the number of amorphous plugs, $\log_{10}$ of the number of filamentous plugs, and the depth in centimeters from the cambium that the sample was taken (table 3). Thus, amorphous plugs alone accounted for 26% of the variability and depth for 12% of the variability. Filamentous plugs made a significant contribution to reduction in water flow but accounted for only 2% of the variability. However, there is a low, positive correlation between the number of amorphous and filamentous plugs. Thus, if filamentous plugs are considered first in a stepwise analysis, they account for 19% of the variability and amorphous plugs account for only 9%. The analysis in table 3 may overestimate somewhat the contribution of the amorphous plugs in reducing water flow.

Only 40% of the variability in water flow was accounted for by the factors included in this analysis. As indicated by previous workers (1, 9), vessel size and distribution probably have significant effects on water flow. Coating of vessel walls with plugging materials could also reduce water flow in the absence of countable plugs.

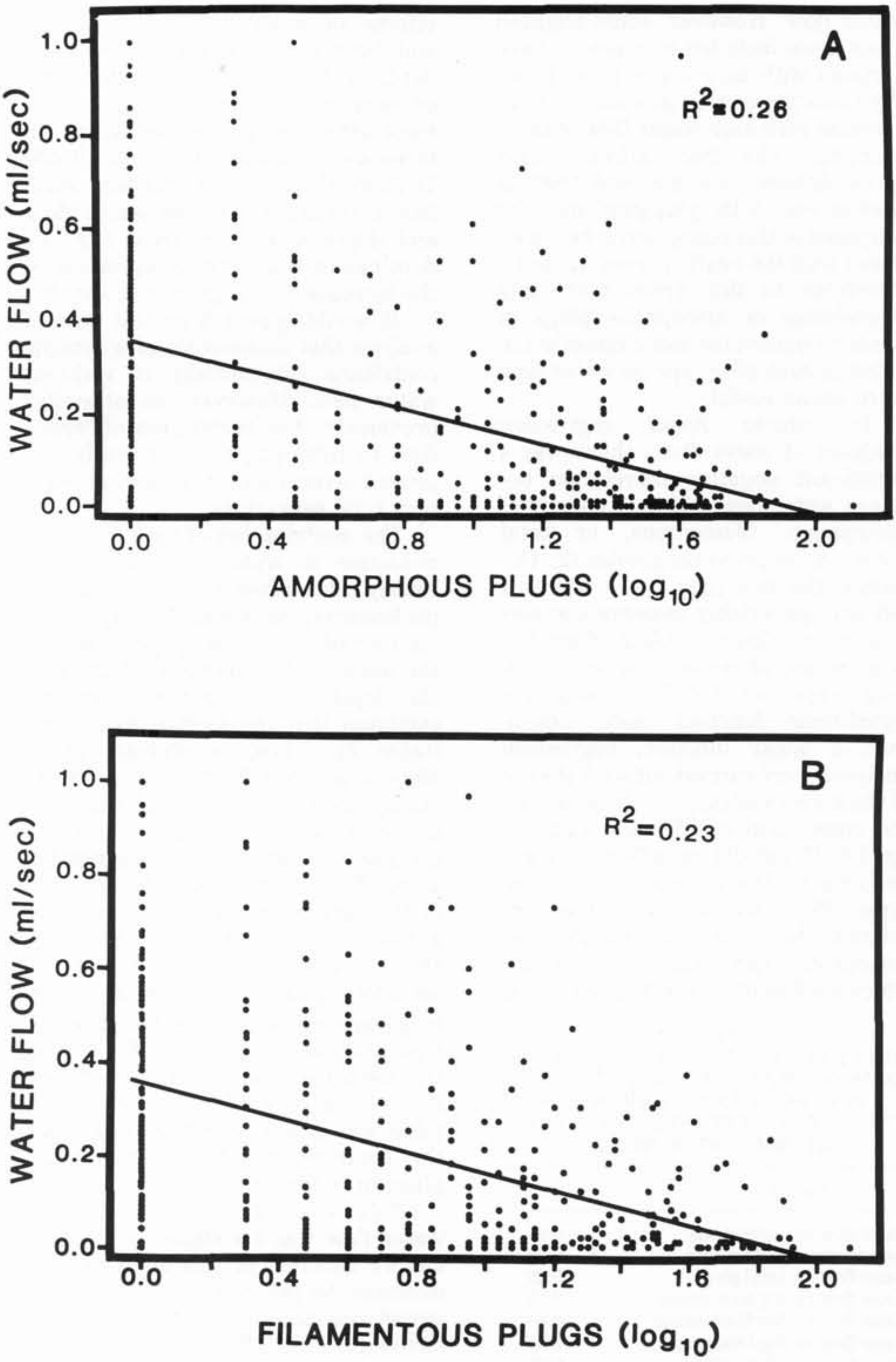

Fig. 1. Relationship of the log of the number of amorphous plugs (A) and the log of the number of filamentous plugs (B) to the water flow through cores of xylem tissue from blight-affected trees, low vigor, apparently healthy trees, Phytophthora and tristeza-affected trees, and from healthy citrus trees.

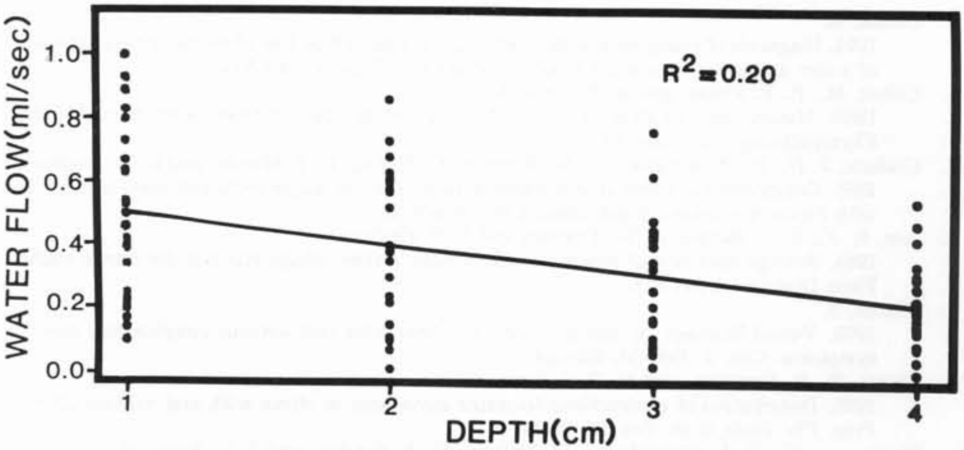

Fig. 2. Relationship of water flow to depth of the sample from the cambium in cores of xylem tissue from the trunks of healthy citrus trees.

It has been apparent from previous work (2, 3, 6, 11, 13) that amorphous plugs occur primarily, if not exclusively, in trees affected by blight and blight-like declines and that amorphous plugs substantially reduce water flow and water uptake. The contribution that filamentous plugs make to reduced water flow has not been clear. It appears from the current work that although filamentous plugs significantly affect water flow, their effect is probably less than that of amorphous plugs.

TABLE 3
STEPWISE MULTIPLE LINEAR REGRESSION OF THE FACTORS AFFECTING WATER FLOW THROUGH XYLEM OF CITRUS TREES

Factor	F value	Partial R^2	Model R^2
Amorphous plugs (log)	156.6[z]	0.26	0.26
Depth (cm)	89.1[z]	0.12	0.38
Filamentous plugs (log)	12.3[z]	0.02	0.40

Model:
Water flow (ml/sec) = 0.55 − 0.135 log amorphous plugs − 0.07 depth (cm) − 0.07 log filamentous plugs

[z]Significant at $P \leq 0.01$.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the capable technical assistance of S. E. Zitko and H. A. Sandler in the conduct of this study. The advice of F. G. Martin, L. W. Duncan, and T. A. Wheaton on the statistical analysis of the data is greatly appreciated.

LITERATURE CITED

1. Albrigo, L. G., J. P. Syvertsen, and R. H. Young
1986. Stress symptoms of citrus trees in successive stages of decline due to blight. J. Amer. Soc. Hort. Sci. 111: 465-470.
2. Brlansky, R. H., L. W. Timmer, R. F. Lee, and J. H. Graham
1984. Relationship of xylem plugging to reduced water uptake and symptom development in citrus trees with blight and blight-like declines. Phytopathology 74: 1325-1328.
3. Brlansky, R. H., R. F. Lee, and M. H. Collins
1985. Structural comparison of xylem occlusions in the trunks of citrus trees with blight and other decline diseases. Phytopathology 75: 145-150.
4. Childs, J. F. L., and T. C. Carlysle
1974. Some scanning electron microscope aspects of blight disease of citrus. Plant Dis. Rep. 58: 1051-1056.

5. Cohen, M.
1974. Diagnosis of young tree decline, blight, and sand hill decline of citrus by measurement of water uptake using gravity injection. Plant Dis. Rep. 58: 801-805.
6. Cohen, M., R. R. Pelosi, and R. H. Brlansky
1983. Nature and location of xylem blockage structures in trees with citrus blight. Phytopathology 73: 1125-1130.
7. Graham, J. H., R. H. Brlansky, L. W. Timmer, R. F. Lee, L. J. Marais, and G. S. Bender
1985. Comparison of citrus tree declines with necrosis of major roots and their association with *Fusarium solani*. Plant Disease 69: 1055-1058.
8. Lee, R. F., L. J. Marais, L. W. Timmer, and J. H. Graham
1984. Syringe injection of water into the trunk: a rapid diagnostic test for citrus blight. Plant Disease 68: 511-513.
9. Nemec, S.
1975. Vessel blockage by myelin forms in citrus with and without rough-lemon decline symptoms. Can. J. Bot. 53: 102-108.
10. Nemec, S., R. Constant, and M. Patterson
1975. Distribution of obstructions to water movement in citrus with and without blight. Proc. Fla. State Hort. Soc. 88: 70-75.
11. Timmer, L. W., R. H. Brlansky, J. H. Graham, H. A. Sandler, and J. P. Agostini
1986. Comparison of water flow and xylem plugging in declining and apparently healthy citrus trees in Florida and Argentina. Phytopathology 76: 707-711.
12. VanderMolen, G. E.
1978. Electron microscopy of vascular obstructions in citrus roots affected with young tree decline. Physiol. Plant Pathol. 13: 271-274.
13. VanderMolen, G. E., R. N. Gennaro, and T. O. Peeples
1975. Chemical nature and statistical analysis of the distribution of plugging in blight/YTD-affected citrus trees. Proc. Fla. State Hort. Soc. 88: 76-79.
14. Wutscher, H. K., M. Cohen, and R. H. Young
1977. Zinc and water soluble phenolic levels in the wood for the diagnosis of citrus blight. Plant Dis. Rep. 61: 572-576.
15. Young, R. H., and S. M. Garnsey
1977. Water uptake patterns in blighted citrus trees. J. Amer. Soc. Hort. Sci. 102: 751-756.