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Xylem Plugging, Hydraulic Conductivity, Growth, and Yield of Citrus Trees Affected by Citrus Declinamiento in Argentina[1]

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ABSTRACT. Citrus declinamiento (CD) is a decline disease similar to citrus blight which has caused severe loss of trees on trifoliate orange rootstock in Misiones, Argentina. Trees on rootstocks other than trifoliate orange do not show decline symptoms, but have reduced water uptake due to amorphous plugs in the xylem and have been designated low vigor trees. Water uptake, transport, and xylem plugging of mature trees on rough lemon, Rangpur, Cleopatra mandarin, and sweet orange rootstocks were followed for 4 yr to determine their effects on trunk growth and yield. Water uptake, as measured by syringe injection into trunks, was greater in trees on sweet orange rootstock than those on other rootstocks, but did not change appreciably from 1984 to 1988 in trees on any stock. Hydraulic conductivity in xylem cores extracted from the trunk also was greatest on trees on sweet orange rootstock and increased during the course of the study. Water flow on a whole tree basis increased from 1984 to 1988 presumably due to the production of more xylem tissue. All trees had high numbers of amorphous occlusions in the xylem, but there were fewer occlusions in the outer centimeter of xylem where hydraulic conductivity was greatest. Trunk growth and fruit yield were greatest in trees on Cleopatra mandarin rootstock. All low vigor trees regardless of stock continued to grow and produce commercial crops through the course of the study in spite of extensive xylem plugging and impaired water transport.
Index words. Citrus blight.

Citrus declinamiento (CD) in Argentina and citrus blight (CB) in Florida are decline diseases which are characterized by xylem blockage, low water uptake and transport through xylem, and accumulation of zinc in trunk phloem and xylem (3, 5, 6, 7, 8, 9, 11, 15). There are many similarities between the diseases, but differences exist in rootstock susceptibility and the age at which trees decline (11, 15). CB has been transmitted experimentally by root grafts (12).

In Florida, trees on rough lemon, Carrizo citrange, Rangpur, and trifoliate orange rootstocks are susceptible to CB and decline rapidly after the appearance of the first symptoms (9, 13). Millions of trees have been lost to the disease since the mid-1960's (13). The disease develops only in older trees on Cleopatra mandarin rootstock and is observed only occasionally in trees on sour orange and sweet orange rootstock (13). In Argentina, trees on trifoliate orange rootstock are highly susceptible and decline and die at an early age (11). The entire citrus industry of the province of Misiones which was based on trifoliate orange rootstock has been eliminated by the disease over the last 25 years and to some extent replaced with trees on other rootstocks (11). To date, most trees on rough lemon, Rangpur, and Cleopatra mandarin rootstocks have survived, seem to be commercially viable, but are not highly vigorous or productive.

In a previous study (10), we described the water uptake, hydraulic conductivity, and xylem plugs in trees in Argentina which we designated as "low vigor" because, while they did not succumb to CD, they were not as vigorous and productive as completely healthy trees. Low vigor trees show no decline symptoms, but have short growth flushes and leaves are often smaller than normal. These low vigor trees had amorphous plugs which were indistinguishable from those in trees affected by CD and CB. They took up more water than CD and CB-affected trees but much less than healthy trees. At that time, questions still remained about the future course of plug development and water transport in these trees and the effect of the reduced water flow on their productivity and longevity. The purpose of this study was to evaluate the potential usefulness of several rootstocks in areas where CD is endemic based on changes in plugging in xylem, water uptake into tree trunks, hydraulic conductivity of trunk xylem, and the trunk growth and productivity of these low-vigor-trees.

MATERIALS AND METHODS

This study was conducted in a rootstock trial located near Eldorado, Misiones, Argentina in which Valencia and Westin sweet oranges on rough lemon, Rangpur, Cleopatra mandarin, sweet orange, trifoliate orange, and Troyer citrange rootstocks were planted in 1972 (1). Rootstocks were arranged in a randomized complete block design with six replications. Each replication consisted of a four-tree plot, two of which were Valencia and two Westin sweet orange. All trees on trifoliate orange rootstock had declined prior to the initiation of this study and many had died.

Six trees each of Valencia orange on rough lemon, Rangpur, Cleopatra mandarin, and sweet orange rootstocks were selected for detailed study of water uptake and xylem plugging. Water uptake into the trunk was measured by the syringe injection method (7) and reported in milliliters per second. Horizontal core samples were taken from the trunk about 30 cm above the bud union with a 5-mm-diameter Haglof increment borer (Forestry Suppliers, Jackson, MS). Cores were fixed overnight in 3% glutaraldehyde in 0.066 M sodium-potassium phosphate buffer, pH 6.8. The glutaraldehyde solution was removed, replaced with phosphate buffer, and the cores stored at 4 C. Water uptake tests were conducted and core samples collected in 1984, 1986, and 1988 in the late summer and early fall after most of the growth for the season was complete.

Hydraulic conductivity in cores was determined for a single core per tree at each date in four 1-cm segments measured from the cambium with a device described and presented diagrammatically elsewhere (2). The apparatus consisted of metal tubing 4.25 mm in diameter 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 4.25-mm-diameter opening was centered over the desired core segment, the core was oriented in the direction of normal water flow, and a vacuum of 91.7 kPa was applied. Water was drawn through each core segment for 30 sec, the flow in milliliters per second recorded, and the hydraulic conductivity in ml/sec/m^2/Pa was calculated.

Trunk circumference 10 cm above the bud union was determined in early 1985 and in early 1989 and trunk cross-sectional area calculated as a measure of tree growth during the test period. Total water flow in the outer 4 cm of each tree was calculated at the outset and at the end of the experiment. To accomplish this, the water flow per core segment was first divided by the cross-sectional area of the core through which water flow was determined (0.196 cm^2) and multiplied by the water flow in the respective core segment to obtain flow in ml/sec/cm^2. This value was then multiplied by the cross-sectional areas of the 0-1, 1-2, 2-3, and 3-4 cm core segments around the entire trunk circumference of the tree. The water flow in each of the four segments was added together to estimate water flow per tree in ml/sec and then divided by the pressure to give water flow in ml/sec/kPa/tree.

After water flow determinations, transverse sections 30 to 50 μm thick were cut from the center one-third of each segment with an AO Spencer Model 860 sliding microtome. The number of amorphous plugs in 200 vessels were counted in random microscope fields at 100X and expressed as percent of the vessels plugged.

Fruit was harvested from the test

at maturity in late winter or early spring (August to November) of each from 1983 to 1988 and yields expressed in kilograms per tree.

RESULTS

Uptake of water by the syringe injection technique was low in all trees tested compared to that in healthy trees in Argentina and elsewhere (3, 6, 7). When compared using an analysis of variance with year and rootstock being considered as independent factors, water uptake was greatest in trees on sweet orange rootstock across all years (table 1). When each year was considered separately, sweet orange again had greater water uptake than any of the other rootstocks in 1984, 1986, and 1988. There was no significant difference between years when water uptake was considered within each rootstock or across all rootstocks.

The hydraulic conductivity of all core segments decreased sharply with increasing distance from the cambium (table 2). Conductivity through core segments of trees on sweet orange rootstock was greater than that of segments from trees on all other rootstocks in nearly every case. Water conductivity across all rootstocks and core segments increased from an overall mean of 0.08 ml/sec/m²/Pa in 1984 to 0.12 ml/sec/m²/Pa in both 1986 and 1988.

The trunk cross-sectional area of trees on all rootstocks increased during the course of the experiment and trees on Cleopatra mandarin had the greatest increase (table 3). When water flow rates per tree were calculated taking into consideration the flow rates and the area of the xylem tissue, water flow rates increased significantly from 1984 to 1988. Generally, trees on Cleopatra mandarin and sweet orange rootstocks had greater total water flow than those on rough lemon and Rangpur.

When analyzed across all core segments, trees on Cleopatra mandarin rootstock had the highest number of amorphous plugs in xylem vessels and those on sweet orange rootstock had the lowest number. The percentage of vessels plugged when averaged across all rootstocks and core segments was 8.6% in 1984, 10.3% in 1986, and 13.9% in 1988. When comparisons were made within each core segment, there was no significant effect of rootstock on amorphous plug counts in core segments 0-1 or 1-2 (table 3). In the third segment, trees on rough lemon and Cleopatra mandarin had the highest percentage of vessels plugged and those on sweet orange the lowest. In the fourth segment, trees on Rangpur and sweet orange had fewer plugged vessels than trees on rough lemon and Cleopatra mandarin.

Yields followed alternate bearing pattern from year to year on all rootstocks. Over the 6 yr in which yield was recorded, only small differences were observed between trees

TABLE 1
EFFECT OF ROOTSTOCK AND YEAR ON THE UPTAKE OF WATER INTO LOW VIGOR VALENCIA SWEET ORANGE TREES USING THE SYRINGE INJECTION METHOD

Rootstock	Water uptake (ml/sec) 1984	1986	1988	Rootstock means
Rough lemon	0.05	0.12	0.09	0.09 b[z]
Rangpur	0.11	0.08	0.17	0.12 b
Cleopatra mandarin	0.07	0.08	0.08	0.08 b
Sweet orange	0.21	0.40	0.29	0.29 a
Year means[z]	0.11	0.17	0.16	

[z]Analysis of variance considering rootstock and year as independent factors. Mean separation by Duncan's multiple range test, $P \leq 0.05$. Year means were not significantly different.

TABLE 2
EFFECT OF ROOTSTOCK AND YEAR OF SAMPLE COLLECTION ON THE HYDRAULIC CONDUCTIVITY OF CORE SEGMENTS OF XYLEM TAKEN 0-1, 1-2, 2-3, AND 3-4 CM FROM THE CAMBIUM OF LOW VIGOR VALENCIA SWEET ORANGE TREES

		Water flow (ml/sec/m²/Pa)			
		Core segment			
Rootstock	Year	0-1	1-2	2-3	3-4
Rough lemon	1984	0.12	0.03	0.02	0.02
	1986	0.23	0.06	0.05	0.02
	1988	0.26	0.06	0.02	0.01
	Mean	0.20 b[z]	0.05 b	0.03 b	0.02 b
Rangpur	1984	0.13	0.04	0.04	0.03
	1986	0.20	0.03	0.02	0.03
	1988	0.33	0.08	0.02	0.02
	Mean	0.22 b	0.05 b	0.03 b	0.03 b
Cleopatra mandarin	1984	0.26	0.08	0.01	0.02
	1986	0.20	0.12	0.06	0.02
	1988	0.24	0.11	0.08	0.04
	Mean	0.23 b	0.10 b	0.05 ab	0.03 b
Sweet orange	1984	0.28	0.10	0.04	0.06
	1986	0.34	0.27	0.14	0.19
	1988	0.37	0.19	0.03	0.07
	Mean	0.33 a	0.19 a	0.07 a	0.11 a

[z]Analysis of variance considering rootstock and year as independent factors. Mean separation within core segment by Duncan's multiple range test, $P \leq 0.05$.

on the different rootstocks (table 5). Yield was greatest on trees on Cleopatra mandarin rootstock and lowest on trees on Rangpur and sweet orange; those on rough lemon were intermediate. Although trees on sweet orange rootstock had the greatest water uptake (table 1), greatest hy-

TABLE 3
EFFECT OF ROOTSTOCK ON THE TRUNK CROSS-SECTIONAL AREA AND ON THE TOTAL WATER FLOW PER TREE OF LOW VIGOR VALENCIA SWEET ORANGE TREES

	Trunk cross-sectional area (cm²)		Water flow/tree (ml/sec/kPa)[z]	
Rootstock	1985	1989	1985	1989
Rough lemon	241 b[y]	299 b	0.88 b	1.56 a
Rangpur	239 b	284 b	1.14 ab	2.05 a
Cleopatra mandarin	325 a	418 a	2.08 a	2.29 a
Sweet orange	252 b	313 b	2.16 a	2.64 a
Mean	264	329*[x]	1.57	2.14*[x]

[z]Calculated by multiplying the flow rate in ml/sec/cm² through the 0-1, 1-2, 2-3, and 3-4 cm core segment by the cross-sectional area of the corresponding segment around the entire trunk circumference and dividing by the pressure used.
[y]Mean separation within years by Duncan's multiple range test, $P \leq 0.05$.
[x]Significantly greater than the 1985 mean, $P \leq 0.05$.

draulic conductivity (table 2), and the fewest amorphous plugs (table 4), they had the lowest yields (table 5).

DISCUSSION

Trees on all rootstocks except those on sweet orange had low uptake and low hydraulic conductivity and all trees had substantial numbers of amorphous plugs. Those on rough lemon, Rangpur, and Cleopatra mandarin rootstocks had greater uptake than trees declining from CB or CD (10), but substantially less than the expected water uptake for normal, healthy trees (6, 7). Despite the presence of large numbers of amorphous plugs in the xylem vessels, water uptake and hydraulic conductivity through core segments was still substantial, but generally not as great as most healthy trees (10).

During the course of the experiment, water uptake when measured by the syringe water uptake test and considered across all rootstocks, showed no significant change from 1984 to 1988. The hydraulic conductivity of core segments increased from 1984 to 1986, but not from 1986 to 1988. The percentage of vessels plugged increased slightly, but consistently, from 1984 to 1988, but this increase was apparently insufficient to affect water uptake or flow greatly. Water flow potential on a whole tree basis increased during the course of the experiment. Thus, even though plugging increased slightly during this time, the trees presumably produced enough new xylem to more than compensate for that which became plugged. Therefore, potential tree water movement has improved during the last 4 yr in spite of the ap-

TABLE 4
EFFECT OF ROOTSTOCK AND YEAR OF SAMPLE COLLECTION ON THE PERCENTAGE OF VESSELS WITH AMORPHOUS PLUGS IN CORE SEGMENTS OF XYLEM TAKEN 0-1, 1-2, 2-3, AND 3-4 CM FROM THE CAMBIUM OF LOW VIGOR VALENCIA SWEET ORANGE TREES

		Vessels with amorphous plugs (%)			
		Core segment			
Rootstock	Year	0-1	1-2	2-3	3-4
Rough lemon	1984	7.9	11.5	20.4	8.5
	1986	5.3	6.8	7.9	16.8
	1988	4.2	16.3	25.6	14.6
	Mean	5.8 a[z]	11.5 a	18.0 a	13.3 a
Rangpur	1984	9.1	8.1	4.5	2.4
	1986	5.5	13.2	10.9	7.3
	1988	7.6	19.1	24.0	11.1
	Mean	7.4 a	13.5 a	13.1 ab	6.9 b
Cleopatra mandarin	1984	3.3	11.9	16.9	12.3
	1986	6.9	15.4	15.6	19.1
	1988	2.6	16.3	19.0	22.7
	Mean	4.3 a	14.5 a	17.2 a	18.0 a
Sweet Orange	1984	2.8	19.8	4.3	2.7
	1986	5.4	14.2	9.7	4.8
	1988	3.0	10.5	10.9	9.9
	Mean	3.7 a	13.2 a	8.3 b	5.8 b

[z]Analysis of variance considering rootstock and year as independent factors. Mean separation within core segment by Duncan's multiple range test, P ≤ 0.05.

TABLE 5
EFFECT OF ROOTSTOCK AND YEAR ON THE YIELD OF LOW VIGOR VALENCIA SWEET ORANGE TREES

	Yield (kg/tree)						
Rootstock	1983	1984	1985	1986	1987	1988	Rootstock means
Rough lemon	114	55	201	121	170	67	121 ab[z]
Rangpur	133	55	162	81	135	75	107 b
Cleopatra mandarin	139	55	204	96	170	131	133 a
Sweet orange	99	52	172	103	126	69	104 b
Year means	121 c[z]	54 e	185 a	100 d	158 b	85 d	

[z]Analysis of variance considering rootstock and year as independent factors. Mean separation for years and rootstocks by Duncan's multiple range test, $P \leq 0.05$.

parent disease condition of these trees.

The appearance of trees on all rootstocks did not change substantially during the course of the study and yields alternated between high and low years as is common for Valencia sweet orange. Normally, trees on Cleopatra mandarin rootstock produce rather low yields, those on sweet orange have moderate yields, and those on Rangpur and rough lemon are vigorous and highly productive (4). Surprisingly, trees on sweet orange rootstock, in spite of greater water uptake, water flow, and less vessel obstruction than trees on the other rootstocks yielded no more than any other rootstock. Sweet orange is highly susceptible to infection by *Phytophthora* spp., but no evidence of foot rot or gummosis was apparent aboveground in any of the trees studied. However, feeder root loss from infection by this fungus could be responsible for the lower yields of trees on this susceptible stock (4). Trunk cross-sectional areas and yields of trees on rough lemon and Rangpur were less than those of trees on Cleopatra mandarin which is in direct contrast to what is normally observed (4). Perhaps the extensive xylem plugging and reduced water flow in the trees on rough lemon and Rangpur rootstocks have limited their vigor, growth, and yields.

Trees in this experiment are now 17 yr old. They continue to produce small crops of fruit but would be commercially viable if planted at an appropriate spacing. In spite of the rather extensive vessel obstruction in many of these trees, water transport is sufficient to maintain tree condition and yield. All four rootstocks studied seemed to be viable alternatives to trifoliate orange rootstocks in Misiones, Argentina in spite of the fact that trees on rough lemon and Rangpur are severely affected by CB and related diseases in other areas of the world. Perhaps, Cleopatra mandarin would be the rootstock of choice for the area at the present given its better performance in this test. Alternatively, growers could plant separate blocks of trees on different rootstocks as a precaution against future problems.

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