Xylem Plugging and Water Flow in Citrus Seedlings and Grafted Plants in Relation to Declinio

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ABSTRACT. Healthy and declinio-affected rootstock seedlings and plants budded to different scions were compared. Water uptake by syringe injection and the number of amorphous plugs in xylem vessels were determined. There were no statistical differences in the number of amorphous plugs between the seedlings of Rangpur lime, Valencia sweet orange and Cleopatra mandarin; the scions and rootstocks of healthy trees on the aforementioned stocks, and the rootstocks of declinio-affected grafted plants. Only the scions of declinio-affected Valencia and Pera sweet oranges on Rangpur lime rootstock showed significantly higher numbers of amorphous plugs. Rangpur lime seedlings and the scions and rootstocks of healthy Valencia sweet orange budded on Rangpur lime rootstock showed normal water uptake, whereas water uptake was very low in rootstocks and scions of declinio-affected Valencia and Pera sweet orange on Rangpur lime rootstock. No water uptake was observed in either the rootstock or the scion of declinio-affected trees, but the number of amorphous plugs was higher only in the scion of declinio-affected trees.
Index words. blight, water uptake, amorphous plugs.

Declinio, a citrus disease similar to blight (1), is one of the most serious production problems of Brazilian citriculture. Declinio and blight are characterized by reduced water uptake in the root and trunk xylem vessels (5, 7, 8, 12), zinc accumulation in the trunk (9, 10, 11) and the presence of occlusions in xylem vessels (1, 4, 5, 7).

Two types of occlusions or plugs, filamentous (4, 5, 8) and amorphous (5, 7), have been observed in the xylem of blight- and declinio-affected trees. Filamentous plugs, consisting of a mass of fine fibers (0.2-0.7 μm in diameter), are usually found in vessels and walls. Amorphous plugs appear to be solid and may completely block the vessel lumen. Brlansky, *et al.* (3) showed that as the number of amorphous plugs in the trunk wood of blight-affected trees increased, water uptake was reduced and the canopy condition declined. No such association existed with the filamentous plugs (3).

Rangpur lime is the most popular citrus rootstock in Brazil. Whereas declinio affects trees budded on Rangpur lime, no declinio in rootstock plants of Rangpur lime has been found in commercial orchards in Brazil. In Florida and elsewhere, blight has affected seedling plants (W. Castle, personal communication, 1986).

The purpose of this study was to compare the water uptake and number of amorphous plugs between mature rootstock plants and healthy and diseased budded trees.

MATERIALS AND METHODS

Comparisons were made between rootstock seedlings (commercial budded plants where the scion had died soon after planting so that the rootstock sprouts developed as a tree) and healthy and declinio-affected, mature commercial trees of different scion-rootstock combinations.

The trees used in this experiment were selected from two orchards, and ranged in age from 12 to 15 yr. The following numbers of seedlings and scion-rootstock combinations were selected for this experiment at the Sete Lagoas Farm: five healthy and five declinio-affected Valencia and Pera sweet oranges on Rangpur lime rootstock; three healthy Valencia sweet orange seedlings; and five Rangpur lime seedlings. At the Nova Era Farm, the numbers were as follows: five healthy and five declinio-affected Valencia and Pera sweet oranges on Rangpur lime rootstock;

ten healthy Valencia sweet orange on Cleopatra mandarin rootstock; ten healthy Cleopatra mandarin seedlings; and five healthy Rangpur lime seedlings.

All trees were tested for water uptake by the injection technique (6). A 3-mm hole, 30 mm deep, was drilled in the trunk 20 cm below and above the budunion on budded plants and 20 cm above the soil on the seedling trees. The time needed to inject 10 ml of water into the trunk using a 20-ml eccentric luertip syringe was recorded, and data were expressed in ml per second. The average pressure applied was about 7.75 kg/cm^2.

Core samples of the trunk wood, 5-7 cm long, were taken from the scion and rootstock of healthy, diseased and rootstock seedlings just above the hole used for the syringe injection test. The horizontal samples were taken with a Hagloff 5-mm diameter increment borer. After removal, the cores were stored in 0.066 M sodium phosphate buffer, pH 6.8 with 0.02% sodium azide until examined. Cross sections, 20 μm thick, were cut from the core at a depth of 2.3 cm from the cambium using a cryostat. Ten sections from each core were mounted on microscope slides, and the number of amorphous plugs in 200 xylem vessels was counted under the microscope in random microscope fields at 100X magnification.

Each part of the plant from which the sample was collected (indicated by the arrows in fig. 1), was considered a treatment. The experiment included 13 treatments with 10 replications (10 trees) of each treatment. For rootstock seedlings of Valencia sweet orange only three replications were used (fig. 1).

The data for the mean percentage of xylem vessels with amorphous plugs and the mean water uptake (ml/second) were submitted to an analysis of variance, as a completely randomized design and then means compared by Tukey's test.

The canopies of healthy and declinio-affected trees were rated visually on a scale of 0 to 3 whereby 0 = healthy and 3 = severe declinio as previously described (6).

RESULTS AND DISCUSSION

All treatments were tested by analysis of variance and the treatment means were compared (table 1). When declinio-affected scions of Valencia and Pera sweet oranges on Rangpur lime were compared, the percentage of vessels with amorphous plugs was different from that of all other treatments and Valencia and Pera also differed. The same comparison was made with the rootstocks of these varieties. Only the scions of declinio-affected plants had a higher percentage of vessels with amorphous plugs when compared with the same healthy combinations. The percentage of vessels with amorphous plugs was greater in diseased scions of Pera sweet orange than in diseased scions of Valencia sweet orange when the rootstock was Rangpur lime.

The scions and rootstocks of healthy Valencia sweet orange on Rangpur lime and Rangpur lime seedlings had satisfactory water uptake, which differed from that of diseased scions and from rootstocks of healthy Valencia and Pera sweet orange on Rangpur lime rootstock where water uptake was low. The seedlings of Cleopatra mandarin and Valencia sweet orange and both the scion and the rootstock of healthy Valencia on Cleopatra mandarin and Pera on Rangpur lime were intermediate in water uptake. These results show that in all declinio-affected plants, there was no water uptake in the scion or in rootstock, whereas in healthy plants water uptake was normal. Water uptake was similar to that of healthy trees of the same varieties in rootstock seedlings.

As shown in table 1, the healthy rootstock seedlings were intermediate in water uptake. Cleopatra mandarin and Valencia sweet orange

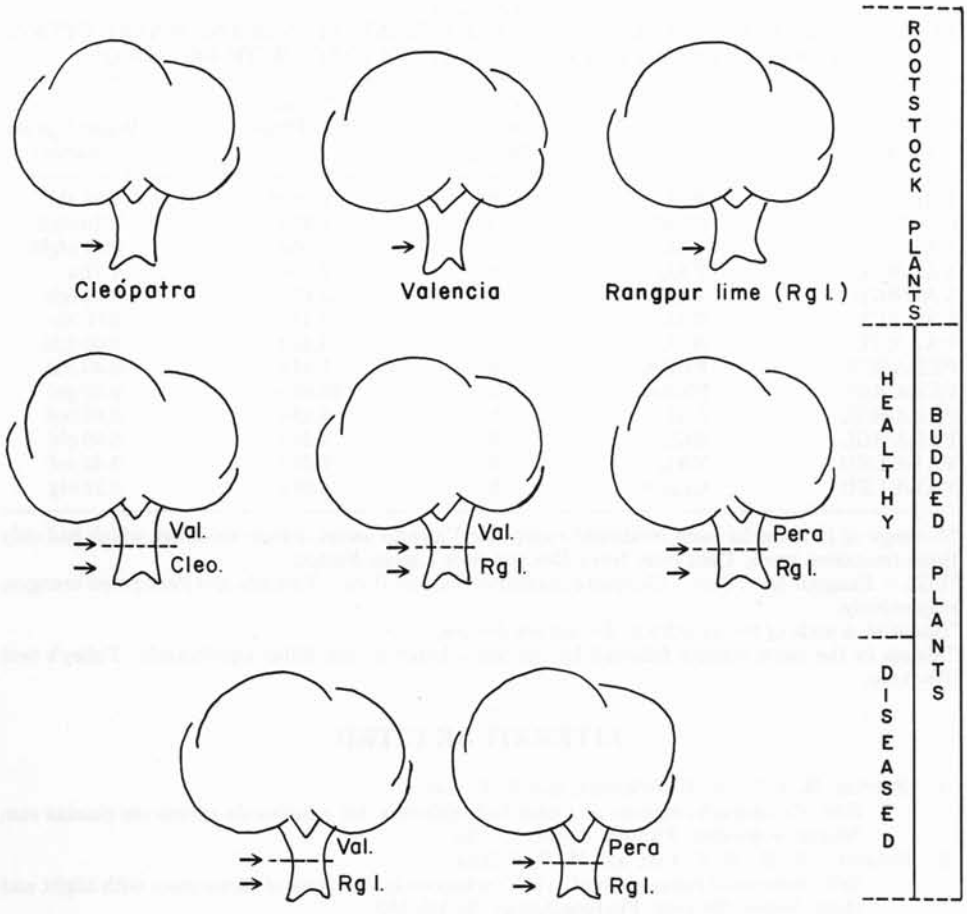

Fig. 1. Diagram of the treatments. Varieties: Pera = Pera sweet orange, Val = Valencia sweet orange, Rgl = Rangpur lime, Cleo = Cleopatra mandarin. → indicates sampling locations (test sites in Table 1).

seedlings had low water uptake, similar to the average amount of water absorbed by diseased trees. In the healthy Valencia sweet orange on Cleopatra mandarin combination, water uptake by the Cleopatra mandarin rootstock was less than that of the Valencia scion. This could be explained by differences in diameter and number of xylem vessels which occur in different varieties.

From this study we conclude that amorphous plugs, characteristic of declinio-affected trees, are observed only in high numbers in the scion of budded declinio-affected trees, and consequently, all those trees suffer a hydraulic deficit and show wilt symptoms. The prevalence of plugs in the scion of declinio-affected trees may give us clues as to the part of the plant to study to better understand this disease. Furthermore, these data suggest that graft incompatibility could be an additional factor involved in declinio.

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TABLE 1
PERCENTAGE OF XYLEM VESSELS WITH AMORPHOUS PLUGS AND WATER UPTAKE IN HEALTHY CITRUS TREES AND CITRUS TREES WITH DECLINIO[z]

Plant[y]	Test Site	Declinio Rating[x]	Vessels with Plugs (%)	Water Uptake (ml/sec)
RGL	RGL	0	0.27 c[w]	1.01 ab[w]
CLEO	CLEO	0	0.23 c	0.10 efgh
VAL	VAL	0	0.95 c	0.07 efghi
VAL/RGL	VAL	0	0.25 c	1.12 a
VAL/RGL	VAL	3	13.07 b	0.01 fghi
VAL/RGL	RGL	0	0.11 c	0.89 abc
VAL/RGL	RGL	3	0.48 c	0.03 fghi
PERA/RGL	PERA	0	1.47 c	0.46 cde
PERA/RGL	PERA	3	24.62 a	0.00 ghi
PERA/RGL	RGL	0	0.18 c	0.66 bcd
PERA/RGL	RGL	3	1.28 c	0.00 ghi
VAL/CLEO	VAL	0	1.23 c	0.42 def
VAL/CLEO	CLEO	0	0.08 c	0.12 efg

[z]Average of 10 trees for each treatment except for Valencia sweet orange seedlings which had only three treatment trees. Data from Nova Era and Sete Lagoas Farms.
[y]RGL = Rangpur lime; Cleo = Cleopatra mandarin; Val and Pera = Valencia and Pera sweet oranges, respectively.
[x]Based on a scale of 0 = healthy to 3 = severe decline.
[w]Means in the same column followed by the same letter do not differ significantly, Tukey's test (P = 0.05).

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