Decline of Citrus Trees in the State of Sao Paulo, Brazil

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A type of citrus decline of unknown cause has been recently described as "declinio" (O. Rodriguez *et al.*, 1979) and is becoming economically important in the State of São Paulo, Brazil. It has been detected in the last 10 years mainly in orchards which have had the best care. Since it occurs in all citrus-growing regions of the State of São Paulo, general quarantine measures within the State have been considered unnecessary. A survey of the occurrence of "declinio" has been carried out in the State of São Paulo and a group has been established to study the disease. Preliminary results are reported in this paper.

Some aspects of "declinio" are similar to citrus blight in Florida (Smith, 1974*a*). Childs (1953) reported the occurrence of obstructions or plugs in the vessels of blight-affected citrus trees in Florida and later (1965, 1979*a*) reported further evidence linking the vessel plugs to blight disease. Plugging in blight-affected trees has been studied also by Nemec (1975), Nemec *et al.* (1975), VanderMolen *et al.*, 1975, and VanderMolen (1978).

Other diagnostic tests for blight, such as water-uptake measurements (Cohen 1974; Young and Garnsey, 1977; Wutscher *et al.*, 1977*b*), zinc and water-soluble phenolic levels in the wood of diseased and healthy trees, and concentration of mineral elements in tissues of affected trees (Smith 1974*b*; Wutscher *et al.*, 1977*a*, 1977*b*; Wutscher and Hardesty, 1979), have been developed in Florida in the last few years. Water-flow rates through some roots of blight-affected trees were also reduced in Florida (Garnsey and Young, 1975).

Results of the experiments conducted in Brazil and Florida to compare blight and "declinio" will be discussed in this paper.

SYMPTOMS

The symptoms of "declinio" occur on bearing trees over 4 years old, frequently after the first crop of fruit. All sweet orange varieties and Tahiti and Galego limes when budded on Rangpur lime and trifoliate orange are susceptible to "declinio." Symptoms have not been observed on mandarins, and it is still uncertain that trees on sweet orange rootstock are affected by "declinio." The first visible symptom, delayed new growth, permits easy detection of diseased trees in orchards in the spring when healthy trees have normal, abundant, new growth. The foliage becomes dull green, followed by wilting of all or part of the canopy and partial or total defoliation and dieback of the trees. Foliar deficiency symptoms of zinc and manganese often occur. Frequently the defoliated trees show abnormal, abundant and vigorous new shoots growing from the junction of the main branches and the trunk. After pruning the outer part of the trees, the new shoots continue growing for some time but the leaves start showing mineral deficiencies and chlorotic patches. When the trunk of declining trees on Rangpur lime is cut below the bud union, apparently healthy shoots of Rangpur lime from the roots are produced in abundance. Declining trees do not recover from the disease, although they may bloom and develop some weak new growth when pruned. Generally, the fruits are smaller than normal, dull and pale green, and yield and juice percentages are reduced. Primary and secondary roots seem normal, except in advanced stages. The number of rootlets is reduced on declining trees. The specific weight of secondary roots of declining trees is less than that of normal trees. Declining trees usually occur in a scattered pattern, rather than in patches.

The symptoms of the disease differ consistently from those described by Rossetti and Namekata (1967) in the Araraquara region of Brazil and from "decaimiento" from Tucuman, Argentina (Rossetti *et al.*, 1972). Symptoms appear to be similar to the type of "definhamento" described for the Cajobi region in Brazil (Rossetti and Namekata, 1967), but no diseased trees were left in that region for accurate comparison. The symptoms also strongly resemble the Misiones "declinamiento" of Argentina, "marchitamiento" of Uruguay (Wutscher *et al.*, 1977*a*), and citrus blight described in Florida.

MATERIALS AND METHODS

Survey. Observations have been made in all the main citrus-growing areas of the State of São Paulo, including Barretos, Bebedouro, Araraquara, Conchal, Limeira, and other locations where large, well-managed groves are flourishing.

Light microscopy studies. Material from both affected and apparently healthy trees from the same orchards was collected. Root pieces, 4 to 6 per tree, were collected at random around the trees approximately 1 meter from the trunk. After removing the bark, pieces 5 to 6 cm long and 10 to 15 mm in wood diameter were fixed in F.A.A. solution No. 2 (100 ml of 50 per cent ethanol, 10 ml of 40 per cent formaldehyde and 10 ml of 100 per cent acetic acid) for 5 or 6 days. Then they were drained and placed damp in plastic bags for air shipment to Florida for observation. The root pieces were trimmed to a length of 40 to 50 mm and longitudinal sections 40 μ thick were cut on a sliding microtome with a freezing attachment. Sections were placed on a microscope slide, flooded with cotton blue stain in lactophenol, covered with a cover slip, and sealed. The mounted specimens were examined with a light microscope using 32 mm objective and 20 X ocular lenses. Plugs were counted and recorded together with the section dimensions. The number of plugs per cubic millimeter was calculated from the data (Childs, 1979*a, b*). Material was thus examined in 1978, especially from orchards of the Barretos and Conchal areas. The data were transformed to log (x + 1) and analyzed statistically (analysis of variance).

Water uptake. In December 1978, water was injected by gravity into the trunks of "declinio"-affected and apparently healthy trees in the field, using Cohen's method (1974) or a more rapid variation of it (Young and Garnsey, 1977). In the Barretos area, 8-year-old trees of Valencia on Rangpur lime rootstock were tested, as well as 6-year-old trees of Natal orange and Tahiti lime on Rangpur. Also, 14- to 15-year-old trees of Valencia and Hamlin oranges on Rangpur lime and trifoliate orange rootstocks were tested in the field in the Conchal area. Data were analyzed statistically using the t test.

Zinc and water-soluble phenolic levels. Wood samples collected from the same trees used for the water-uptake tests, and from trees in other locations in 1977 and 1978, were analyzed for zinc and magnesium by atomic absorption, for potassium by flame emission, and for water-soluble phenolics with a UV spectrophotometer (Wutscher *et al.*, 1977*b*). The t test was used in Orlando, Florida to analyze the results.

Waterflow rates. Using the method described by Bitancourt *et al.* (1943) for studies in wood alterations due to psorosis, and later by Garnsey and Young (1975) for blight, three experiments were carried out in the laboratory to measure the ability of roots to conduct water. Root pieces 6 cm long and 10 to 15 mm in diameter were connected to a vacuum source and to a calibrated water reservoir. The end of the root closest to the trunk was connected to the vacuum source so that the water movement was in the direction of normal flow. Vacuum of approximately 400 mm Hg was applied by an electric pump. Two root pieces, one from declining trees and the other from nearby, apparently normal trees with cross-sectional areas as similar as possible, were tested at the same time. The cross-sectional area was calculated by measuring the diameter of the root pieces or of the woody portion of the roots. Root specimens were collected in the afternoon, kept moist in the refrigerator, and tested the next day after warming to room temperature. Flow rate was calculated as ml per cm^2 of xylem cross-sectional area per minute to correct for differences in root size. In the first and second experiments, roots of two groups of ten 8-year-old trees of nucellar Valencia on Rangpur lime rootstock were tested (five declining and five apparently healthy trees from the same orchard of the Barretos region in each group). In the third experiment, two pairs of 14-year-old trees (declining and apparently healthy) from the Conchal region of each of the following four combinations were used: Valencia on Rangpur lime and on trifoliate orange, Natal on Rangpur lime, and Hamlin on trifoliate orange. Four 12-cm-long pieces of roots of each tree in the third experiment were also used for dye absorption, using the method described above, to permit visualizing the pattern of waterflow. A 0.5 per cent water solution of crystal violet was used for this purpose (Young and Garnsey, 1977). Dye absorption was estimated by four ratings from 0 to 3. Results of the three experiments were statistically analyzed using the F test for the first two tests and Tukey's test for the third test.

Airflow rates. Bitancourt *et al.* (1943) measured the airflow through the wood and bark of branches with psorosis lesions and showed that more air passed through the wood in the area of the bark lesions than through healthy tissue. The same principle was used to measure airflow through the wood of root pieces of trees affected by "declinio." Root pieces were collected in an orchard of the Conchal region from two affected and two healthy trees of each varietal combination as described for waterflow measurements. Four root pieces of each tree, 8 cm long and 10 to 15 mm in diameter, were tested. Air flowing through the wood of root pieces was measured by connecting the end of a root piece closest to the trunk to an electric air compressor. A 60-cm-high graduated glass column, 3.8 cm in diameter, was placed vertically with the bottom end immersed in a water reservoir so that it could be filled with water from the upper end. The root piece was placed into the water reservoir with its free end in the bottom part of the column. Compressed air was forced through the root piece, and the bubbles emerging were caught in the column. The volume of water displaced from the column during 30 seconds' operation gave the airflow measurement. The air rate was calculated as ml per cm^2 of root cross-sectional area per minute.

RESULTS

Survey. In the Barretos area, two orchards of Valencia on Rangpur lime with about 12,000 trees each, planted in 1970 with plants of the same origin, were affected by "declinio." One showed 1 to 2 per cent, and the other approximately 20 per cent declining trees. In other orchards, 5.4 per cent of 30,500 6-year-old Natal orange trees, 3.0 per cent of 12,000 7-year-old Tahiti lime trees, 6.7 per cent of 51,800 8-year-old Pera orange trees, and 5.0 per cent of 15,000 Hamlin orange trees, all on Rangpur lime rootstock, have declined. About 20 per cent of Pera orange trees on Rangpur have declined in the Terra Roxa area. In the Conchal area, about 3 per cent of 95,000 14- to 15-year-old orange and lime trees on Rangpur lime and trifoliate orange rootstocks are affected. Eight per cent of 40,000 10-year-old Galego lime trees in the Bebedouro area show decline. Many orchards are still free of decline, however. This variable percentage of declining trees is found in all citrus-growing areas of São Paulo State. Soil and leaf analyses have not detected any differences in nutritional conditions between healthy and "declinio" trees.

Light microscopy studies. Root specimens from 26 trees, 16 declining and 10 apparently healthy of different varieties and from various regions, were collected from March to July 1978 and sent to Florida. Significantly more plugs/mm^3 were found in the roots of declining trees than in healthy trees (F test, P = .01) and the number of plugs per mm^3 was higher in samples from Conchal than in those from Barretos (table 1).

Water uptake. Eight-year-old Valencia and Natal orange, and Tahiti lime trees on Rangpur rootstock in Barretos responded to the water-uptake test in the same way as blight-affected trees in Florida. Water uptake was 8 to 14 times higher in healthy trees. Mean differences were statistically significant at the 1 per cent probability level using the t test. In Conchal, 14-year-old Valencia trees on Rangpur and trifoliate rootstocks with "declinio" absorbed 4 to 12 times less water than the healthy controls, but absorption by Hamlin on these rootstocks was not reduced.

Zinc and water-soluble phenolic levels. In Barretos, other blight tests on trees on Rangpur and trifoliate rootstocks used for water-uptake tests yielded results similar to blight-affected trees in Florida. Zinc in the wood of sweet orange on Rangpur lime was up to four times higher and the phenolics were also consistently higher in declining trees. Mean differences were highly significant at the 1 per cent probability level. Declining Tahiti lime trees on Rangpur rootstock showed increased phenolic levels, but did not show zinc accumulation. Zinc concentration and the water-soluble phenolics levels in the outer layers of the trunk of declining trees were always higher in material collected in 1977 from trees of different varieties in other regions of São Paulo State (table 2). Higher levels of potassium and magnesium in the wood of declining trees were found, except for Tahiti lime trees. The differences were significant at the 1 per cent probability level.

Waterflow rates. Experimental results of waterflow rates through root pieces from trees affected by "declinio" and healthy trees are shown in table 3. In the first and second experiments, waterflow was 49 and 45 per cent less (P = .01) in roots of declining trees than in those of healthy trees. The third experiment gave the same results (P = .05) and also showed significant differences between varietal combinations. Waterflow in all combinations averaged about 60 per cent less in roots of declining trees (table 3). The dye pattern rated 0 to 1 on root pieces of diseased trees of the third experiment and 2 to 3 for those of healthy trees.

Airflow rates. The xylem blockage of airflow was even more drastic than that of waterflow at 25°C. The average airflow rates through root pieces of healthy (H) and diseased (D) trees were, respectively, 1176 and 250 ml per cm^2 of wood sectional area per minute. The average ratio D/H was 0.212.

DISCUSSION AND CONCLUSIONS

In orchards of the Conchal region, an increase of "declinio" disease has been observed mainly on trees of sweet orange on Rangpur lime and trifoliate rootstocks. On sweet orange rootstocks, there is little incidence of the disease and it is uncertain whether this is the same type of decline.

The visual symptoms of most types of decline are not specific. For this reason, a number of diagnostic testing methods which have been developed for blight disease in Florida were applied for "declinio" in Brazil, permitting comparison of both abnormalities.

In both diseases, injuries to the root system, usually found in other diseases involving water-stress symptoms (Rossetti and Namekata, 1967; Cohen, 1974), are not detected. Apparently identical obstructions of xylem vessels have been found in both diseases. A dysfunction of the water-transport system within the

TABLE 1

AVERAGE NUMBER OF PLUGS PER mm^3 FOUND IN VESSELS OF DECLINING (D) AND APPARENTLY HEALTHY (H) TREES IN THE STATE OF SAO PAULO IN 1978

Date	Location	Variety	Condition	No. plugs/mm^3/ no. samples	Mean*
March 22	Conchal	Valencia/Rangpur	D	23.30/7	
March 22	Conchal	Valencia/Rangpur	D	14.50/5	
March 22	Conchal	Valencia/Rangpur	D	15.00/6	17.60
March 22	Conchal	Valencia/Rangpur	H	1.10/6	1.10
March 22	Conchal	Hamlin/trifoliate	D	26.10/4	
March 22	Conchal	Hamlin/trifoliate	D	42.30/4	
March 22	Conchal	Hamlin/trifoliate	D	110.80/5	
March 22	Conchal	Hamlin/trifoliate	D	58.40/6	59.40
March 22	Conchal	Hamlin/trifoliate	H	0.60/7	
March 22	Conchal	Hamlin/trifoliate	H	22.50/6	11.50
May 3	T. Roxa	Pera/Rangpur	D	7.90/5	7.90
May 3	T. Roxa	Pera/Rangpur	H	1.50/5	1.50
May 3	Barretos	Tahiti/Rangpur	D	7.10/5	
May 3	Barretos	Valencia/Rangpur	D	0.34/4	
May 3	Barretos	Valencia/Rangpur	D	2.23/4	
May 3	Barretos	Bahianinha/Rangpur	D	12.50/4	5.54
May 3	Barretos	Bahianinha/Rangpur	H	0.10/5	
May 3	Barretos	Valencia/Rangpur	H	0.86/6	
May 3	Barretos	Tahiti/Rangpur	H	0.32/4	0.43
July 5	Barretos	Valencia/Rangpur	D	17.73/6	
July 5	Barretos	Valencia/Rangpur	D	5.50/6	11.62
July 5	Barretos	Valencia/Rangpur	H	0.00/6	
July 5	Barretos	Valencia/Rangpur	H	0.74/5	0.37

* Data transformed to log (x + 1) for analysis; F (disease) = 25.80, F (location) = 10.23, P = .01; interaction disease x location not significant.

TABLE 2
ANALYSIS OF WOOD SAMPLES COLLECTED AT DIFFERENT LOCATIONS IN THE STATE OF SAO PAULO, BRAZIL

Date	Location	Variety*	Age (yrs)	No. trees	Zinc (ppm) H	Zinc (ppm) D	Phenolics (mg/g) H	Phenolics (mg/g) D	Mg (%) H	Mg (%) D	K (%) H	K (%) D	Ca (%) H	Ca (%) D
4/ 5/77	Conchal	orange/1	10-12	7	8.5†	11.8†	3.2	4.8	—	—	—	—	—	—
8/ 9/77	Conchal	orange/2	10-12	8	5.5	7.5	3.0	4.3	.037	.053	.216	.264	.375	.397
12/27/77	S. Pedro	orange/3	8	3	—	9.0	—	6.6	—	.057	—	.271	—	.170
		lime/4	8	2	—	5.0	—	4.9	—	.030	—	.290	—	.318
12/18/78	Barretos	orange/5	6-8	7	5.3	12.4	3.8	6.0	.053	.055	.171	.325	.297	.279
		lime/6	6	2	3.0	4.0	3.7	7.1	.063	.030	.285	.295	.317	.416
12/19/78	Conchal	orange/7	14-15	5	6.0	8.0	6.0	8.0	.038	.051	.160	.227	.365	.512

* Variety/rootstock = (1) Valencia on Rangpur lime; Hamlin on trifoliate; (2) Valencia on Rangpur lime; (3) Pera on Rangpur lime; (4) Tahiti lime on Rangpur lime; (5) Valencia and Natal on Rangpur lime; (6) Tahiti lime on Rangpur; (7) Valencia on Rangpur lime and trifoliate; Hamlin on Rangpur lime and trifoliate.

† Contamination by Zn from drill.

TABLE 3

WATER FLOW RATES IN ROOT PIECES FROM TREES AFFECTED BY "DECLINIO" (D) AND HEALTHY (H) TREES FROM TWO LOCATIONS IN THE STATE OF SAO PAULO, BRAZIL

Exp.	Scion/stock	Age (yrs)	Location	No. trees	Waterflow rates* $ml/cm^2/min.$ H	D	Flow ratio D/H	Means statistical significance†
1st	Valencia/Rangpur	8	Barretos	5	21.3	10.9	0.51	+ +
2nd	Valencia/Rangpur	8	Barretos	5	12.5	6.9	0.55	+ +
Mean			Barretos	5	16.9	8.9	0.53	
3rd	Valencia/Rangpur	14	Conchal	2	70.7	35.1	0.50	c
	Natal/Rangpur	14	Conchal	2	40.1	7.8	0.19	a
	Hamlin/trifoliate	14	Conchal	2	41.1	18.6	0.45	a, b
	Valencia/trifoliate	14	Conchal	2	59.8	22.8	0.38	b, c
Mean			Conchal	2	53.2	21.1	0.40	

* Four root pieces tested per tree, 6 cm long, 1 to 1.5 cm diameter. For 1st and 2nd exp., total cross-sectional area of roots was considered; for the 3rd exp., only the cross-sectional area of the wood portion of roots was considered.

† + +, F test significant at 1 per cent level; data reduced to $\sqrt{x}$.

a, b, c — Tukey 5 per cent level test. DMS = 0.9821 for data transformed to $\sqrt{x}$.

tree, in the trunk and in the roots seems to be associated both with blight and "declinio," as shown by experiments on water uptake in the trunk, and on water-flow rates in the roots. In Brazil, it has been shown that xylem obstruction or dysfunction can also block airflow through the roots.

The zinc concentration (Smith, 1974*b*) and water-soluble phenolics levels in the outer layers of the trunk wood are higher in affected trees than in healthy trees for blight and "declinio." Higher levels of potassium and magnesium have also been found in the wood of trees affected by both diseases.

Repeated attempts to transmit and perpetuate blight and "declinio" by budding or grafting have been unsuccessful. Soil and leaf analyses have not given any indication as to which elements may be responsible for nutritional disturbances. Even in the Barretos area, where two comparable orchards show a conspicuous difference in percentage of declining trees, one with 1 to 2 per cent and the other with 20 per cent, nutritional differences could not be found.

Studies are underway to compare blight with other types of decline in many countries besides Brazil (Wutscher *et al.*, 1977*a*; Childs, 1979*b*). Similarities have been found with "marchitamiento" in Uruguay, "declinamiento" in Argentina, and similar declines in other countries.

The chemical nature of plugging in blight-affected trees has been studied in Florida (Nemec, 1975; Nemec *et al.*, 1975; VanderMolen *et al.*, 1975). Similar studies are underway for "declinio" in Brazil.

The results of the experiments discussed in this paper give no clear indications as to the causal agent of "declinio," but they show that disturbances of the mineral element metabolism and xylem obstruction and dysfunction are similar in "declinio" and blight.

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