

Citrus Declinio in the State of Bahia, Brazil: Occurrence and Responses to Blight Diagnostic Tests¹

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ABSTRACT. A disorder of citrus trees of undetermined cause, involving wilt, defoliation and general decline, has been observed in the State of Bahia, Brazil since 1970. Recent studies, using tests considered diagnostic for citrus blight in Florida, indicated the disease could be classified into two types: a typical blight or "declinio" type (CDB-T) and an atypical type (CDB-A). While both showed reduced water uptake and hydraulic conductivity rate, relatively high levels of water-soluble phenolics, potassium, and a greater number of vessel plugs than in healthy trees, only CDB-T-affected trees had higher zinc in outer trunk wood tissues. Attempts to determine any involvement of fungi, bacteria, and viruses in the occurrence of either disorder, and applications of growth-promoting substances, organic and inorganic fertilizers, mulching, scion-rooting, and severe pruning to induce tree recovery from decline symptoms have been unsuccessful.

"Declínio" (hereafter referred to as CD) is a term given to describe a citrus disorder occurring in most Brazilian orchards, 4 years or older, manifesting partial or total wilting of the canopy followed by progressive leaf drop and twig dieback. The disorder has been observed in the State of Bahia in 1970 (15), in São Paulo a year or so later (14), and in Sergipe in 1980 (1). CD is becoming Brazil's most serious citrus production problem. Its etiological agent has not yet been identified, while the areas and the number of affected trees are increasing (10). An estimated 10 million trees have already been rendered unproductive or removed due to the disease.

Recent studies (16, 21) showed that trees with CD in São Paulo had restricted water movement in the roots and the trunk, large numbers of vessel plugs in roots, and high levels of zinc and water-soluble phenolics in outer trunk wood, much like trees affected by "declinamiento" in Argentina,

"marchitamiento repentino" in Uruguay, and blight in Florida. Hence, the disorder was considered closely related if not identical. This study was conducted to establish the relationships of CD-like disease observed in the State of Bahia (CDB) since 1970 (15) employing diagnostic tests developed by Wutscher *et al.* (21).

MATERIALS AND METHODS

Several citrus orchards located along Bahia's "Reconcavo" region were inspected for the occurrence of declining trees (fig. 1). The disease incidence in each orchard was determined by counting the number of affected trees in three to five 5-row plots selected at random, depending on the size of the orchard. The rate of the spread of this disorder was determined in the same manner, but in a selected orchard. The same plots were observed yearly. In all cases, water damage, foot rot, psorosis, exocortis, and xyloporosis infected trees were carefully excluded from the survey.

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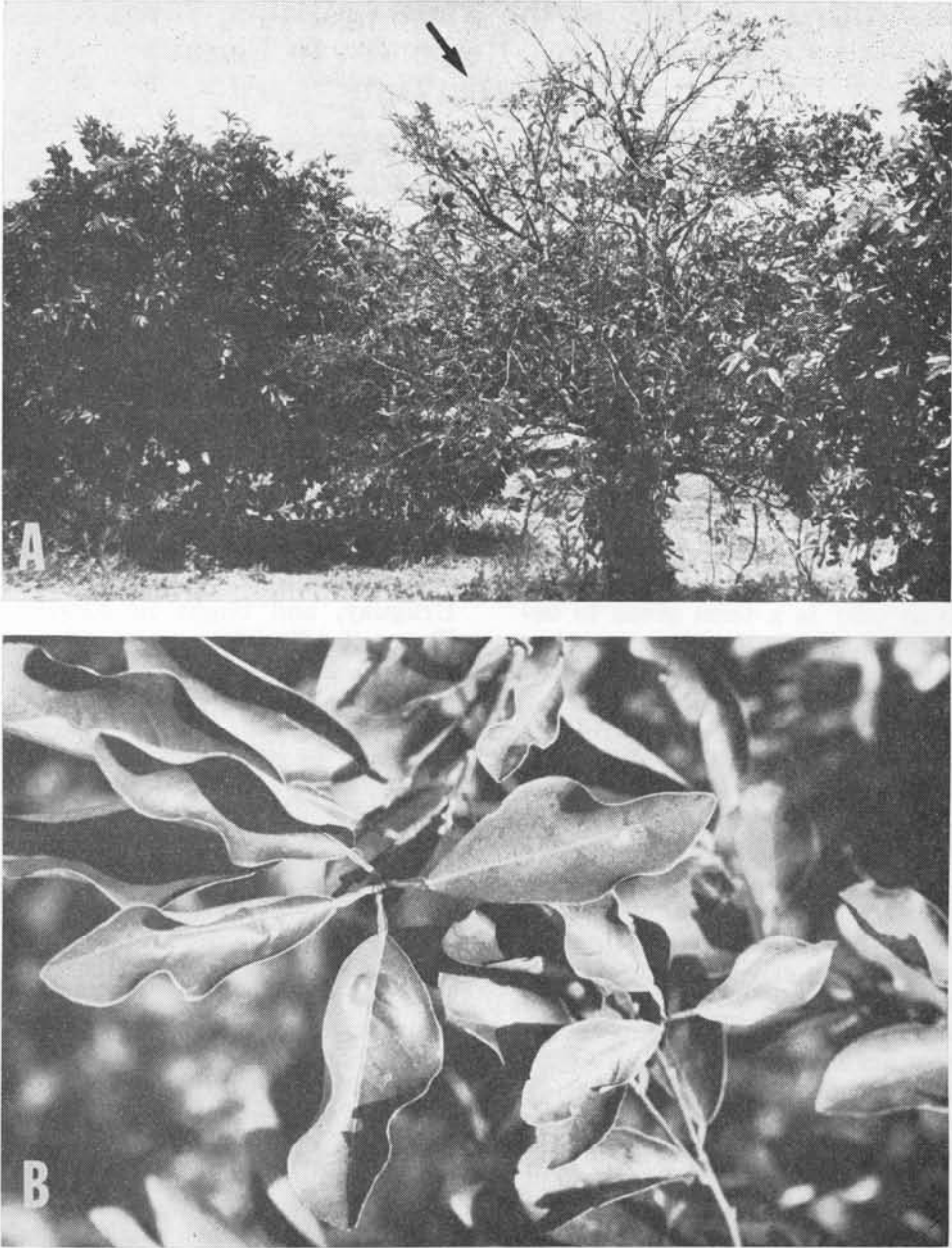


Fig. 1. Pera sweet orange on Rangpur lime rootstock showing severe decline symptoms: A) tree showing severe twig dieback and vigorous shoots from rootstock; and B) dull green, wilted leaves.

Cultivars surveyed were Bahia, Baianinha and Pera sweet orange on Rangpur lime rootstock.

To determine the relationship of CD to blight in Florida, 19 trees with typical CD symptoms and 19 apparently healthy trees from the

same area were subjected to the following tests:

Analysis for zinc and water-soluble phenolics. With the aid of a brace drill, two 13-mm-wide by 25-mm-deep holes were drilled on opposite sides of the trunk, 200 mm

above the budunion. Wood chips from the drilling were collected after the bark had been removed. The chips were dried at 60-65 C overnight and sent to Orlando, Florida, where 2-g samples were dry-ashed at 470 C for 8 hours, and the ash dissolved in 25 ml of 0.6 N HCl. Zinc and potassium levels were determined by atomic absorption and flame emission, respectively. Water-soluble phenolics extracted from 100 mg of wood with 20 ml water were determined spectrophotometrically at 277 nm, using tannic acid as standard (20, 21).

Water absorption by gravity injection. One of the holes drilled to sample the wood above was deepened to approximately 50-60 mm and a calibrated 1500-ml plastic bottle was connected to it with rubber tubing and a short metal connector (3). The water absorbed by the trees during a 24-hour period was noted.

Hydraulic conductivity test. One healthy and two declining trees were selected and tested. Their water uptake and zinc level in outer wood were: healthy, 254 ml and 5.3 ppm; CDB-T, 0 ml and 14.0 ppm; and CDB-A, 12 ml and 4.3 ppm, respectively. The trees were uprooted and 30-mm disks were cut immediately from below the budunion, at the budunion, 200 and 600 mm above the budunion, and from the principal roots and branches of each tree (average trunk diameter 120 mm). The discs were sealed in plastic bags and stored in a freezer. Wood pegs of 9 mm in diameter and 20 mm long from the center, the inner or intermediate wood, and 5 mm from cambial face of each disc were punched out, and their hydraulic conductivity was measured by the method of Cohen (4).

Histological studies. After determining the hydraulic conductivity, pegs were immediately fixed in

formaldehyde alcohol-acetic acid (FAA solution (40% formaldehyde, 50% alcohol, and acetic acid 1:1:1 v:v:v)). The pegs were further trimmed to 5-6 mm diameter before they were sectioned (28-30 μ) in a Jung 1295 freezing microtome. The sections were progressively stained with hematoxylin (2.5% in 95% ethanol) and mounted on microscope slides for examination for the presence of xylem plugs (Cohen, personal communication).

Although fungi, nematodes and viruses had not been directly involved with either "declínio" in São Paulo or blight in Florida (7, 11), this does not mean these pathogens could not be important in the case of CD in Bahia. Hence, that possibility was investigated. Two buds from declínio-infected trees were grafted to each of six seedlings of the following cultivars: sweet orange cvs. Madam Vinous, Pineapple, Caipira, and Persia; grapefruit cv. Duncan; mandarin cv. Parsons Special on rough lemon; Citron Arizona 861; Mexican lime; Eureka lemon; sour orange; rough lemon, Rangpur lime, Volkamer lemon; tangelo cvs. Lee, Minneola, Orlando, Osceola and Sampson; and citrange cvs. Carrizo and Rusk. Three seedlings from each cultivar were grafted with two buds from a healthy tree to serve as controls. All plants were kept under observation in a partially shaded greenhouse (24-32 C) for 2 years. They were pruned occasionally to force development of new shoots. Enzyme-linked immunosorbent assays (ELISA) and starch tests (6) were performed to detect the presence of tristeza virus.

For isolation of fungi, root and bark pieces were collected from diseased trees, surface sterilized and plated on potato dextrose agar. The fungi were identified, and their pathogenicity tested.

Species of nematodes and their populations were determined on 10 healthy and 10 decline-infected Pera sweet orange on Rangpur lime. Four root and soil samples were collected at random around the drip line of the canopy of each tree. The samples were sent to FMC do Brasil S.A., Campinas, São Paulo, where nematodes were isolated by centrifugation flotation method (8) and identified.

In attempts to reverse symptoms, growth regulators (naphthaleneacetic acid (50 ppm), gibberellic acid (100 ppm), 2,4-D (24 ppm), Promalin (45%) and gibberellic acid (100 ppm) + 2,4-D (24 ppm)); organic and inorganic fertilizers; humus; plastic mulch; and severe pruning + scion rooting were tested. A 7-year-old orchard with 5.3% infection was selected for the study. Three trees showing similar degrees of decline were used per treatment. Application of materials were made as follows: growth regulators by spraying 4 liters of solution plus an appropriate amount of wetting agent per plant monthly for 4 months; organic matter and humus, 200 kg each incorporated into the soil; inorganic fertilizer spray applications every 15 days of 10-10-10 liquid fertilizer containing B, Mo, Mn, Fe, Cu, Co, Zn, S (200 ml of fertilizer + 6 ml wetting agent + 20 liters water) for 3 months; and pruning and scion rooting according to Schwarz *et al.* (17).

RESULTS AND DISCUSSION

Distribution and incidence of the disorder. Occasional surveys conducted between May 1981 and March 1983 showed that CD-like disease is widely spread in the "Reconcavo" area of Bahia. Its incidence is still considered low (1.6%), however. The disorder was observed in five of 12 orchards with the following incidence: Conceição

de Feira (1 orchard)—8.4%, Cruz das Almas (2 orchards)—0.2%, Sapeaçu (1 orchard)—0.1%, and Santo Antonio de Jesus (1 orchard)—10.0%. Declining trees were usually distributed throughout the orchard at random, except in Santo Antonio de Jesus where diseased trees were observed in a 3-ha old fertilizer test. The fertilization experiment was abandoned in 1979 due to occurrence of CD-like disease. It is possible that the tree decline observed there may have been caused by nutritional problems.

Rate of decline. The orchard in Conceição de Feira was selected for this study. It was planted in 1975 to Bahia (21 ha), Baianinha (17 ha), and Pera (5 ha), all on Rangpur lime rootstock. When it was first surveyed in March 1982, disease incidence was 5.1, 5.9 and 4.3%, respectively. A year later, 8.0, 10.1 and 7.9% were affected, an average buildup of 3.6% a year.

Again, declining trees were randomly distributed throughout the orchard. There was no indication of increased numbers of trees with CD-like symptoms near the edge or any other part of the orchard, suggesting that the disease may have originated in the area itself. On the other hand, incidence of 3.6% a year does not indicate spread.

CDB diagnosis. The water uptake, zinc, water-soluble phenolics, and potassium concentrations in outer trunk of representative trees with typical decline symptoms from Santo Antonio de Jesus, Cruz das Almas, and Conceição de Feira were compared with those of corresponding healthy trees from the same orchard. The results (table 1) showed that water uptake of declining trees from Cruz das Almas and Conceição de Feira was significantly (<1%) less than that of healthy-appearing trees. Trees in Santo Antonio de Jesus, however,

TABLE 1
 WATER UPTAKE AND ZINC, WATER-SOLUBLE PHENOLICS AND POTASSIUM
 CONCENTRATIONS IN HEALTHY (H) AND DECLINING (D) SWEET
 ORANGE TREES ON RANGPUR LIME IN THREE MUNICIPALITIES OF
 BAHIA, BRAZIL

Location and cultivar	No. of trees	Water uptake (ml/24 hr)	Zinc (ppm)	Phenolics (mg/g)	Potassium (%)	CDB-T/CDB-A† ratio
Santo Antonio de Jesus Baianinha	6 H	213.3	5.9	3.8	0.22	—
	6 D	56.3	6.4	3.9	0.23	0
Cruz das Almas Pera	5 H	170.0***‡	5.6	2.7***‡	0.16**	—
	5 D	18.6	8.0	3.7	0.28	0.2
Conceição de Feira Baianinha	3 H	423.0***	4.0	—	0.08	—
	3 D	15.0	16.7	—	0.12	0.7
Bahia	5 H	548.2***	6.0	2.7**	—	—
	5 D	4.6	10.2	3.6	—	0.4

† Ratio between declining trees with high (CD-T) and low (CD-A) levels of zinc in outer wood.

‡ *** = significant at 0.1% levels; ** = significant at 1% level.

did not show significant differences because two of the suspected diseased trees took up more water than their healthy counterpart.

Water-soluble phenolics in trunk wood of CDB-affected trees from Cruz das Almas and Conceição de Feira were significantly higher than in healthy trees, but this was not true for trees in Santo Antonio de Jesus. Potassium accumulated only in wood of affected trees in Cruz das Almas (table 1).

Generally, zinc concentrations in the outer trunk wood of CDB-affected trees did not differ significantly from those of healthy trees. Nevertheless, five of these trees (1 from Cruz das Almas and 4 from Conceição de Feira) had at least twice as much zinc as healthy trees. Because these trees also exhibit other characteristics (reduced water uptake, and higher water-soluble phenolics and K) typical of blight, they were considered as closely related to blight and designated as CDB-T to distinguish them from trees with similar characteristics but with low zinc (CDB-A) for subsequent studies.

Occurrence of CDB-A is not restricted to the State of Bahia. Trees with similar characteristics had been observed in São Paulo (unpublished data), Florida (20, 22), and Argentina and Uruguay (19, 21).

Cohen (4, 5) reported that reduced water uptake in blight-affected trees could have been due to the inability of water to pass through the trunk-wood conducting vessels. Conductivity of wood pegs 5 mm from the cambium layer of sick trees was similar to that of healthy trees in Florida. Trees with both CDB-T and CDB-A contain plugs. The pattern of conductivity between healthy and declining trees was very similar, but the rate was different. Wood from roots, trunk and branches, and different parts

of the cross-sectional area (center to cambial layer) of affected trees conducted significantly less water than wood from the healthy-appearing tree (table 2). Between the two declínio types, trees with CDB-A appeared to be less conductive than CDB-T-affected trees. No or restricted conductivity was observed in the center of the trunk of both healthy and diseased trees and trunk wood between the center and the cambial layer of both disease types (table 2).

Microscopic examination of stained wood tissues from healthy and declining trees (T and A) revealed the presence of vessel obstructions or filamentous plugs (fig. 2) similar to those described by Nemeč *et al.* (12), Childs (2), and Cohen (personal information) for blighted trees. However, the number of plugged xylem vessels from the healthy trees was significantly less than in CDB-T and CDB-A trees (table 3). On the other hand, no consistent pattern in number of plugs was observed between T and A. They appeared to be well distributed in the affected trees. In healthy tissues, plugs were found mostly in the center of the trunk and the wood 20 cm above the budunion. These observations may explain the reduced water uptake exhibited by the declínio (T or A) affected trees and the close relationship with blight.

Absence of virus involvement. Seedlings of several citrus cultivars inoculated with budwood from CDB-T, CDB-A, and healthy-appearing trees grew normally, except Mexican lime, Eureka lemon, sour orange, and Duncan grapefruit seedlings. They exhibited leaf symptoms characteristic of tristeza virus (CTV) infection. The presence of CTV in healthy and declining trees was also verified by ELISA and starch tests (6). This is expected, because CTV is omni-

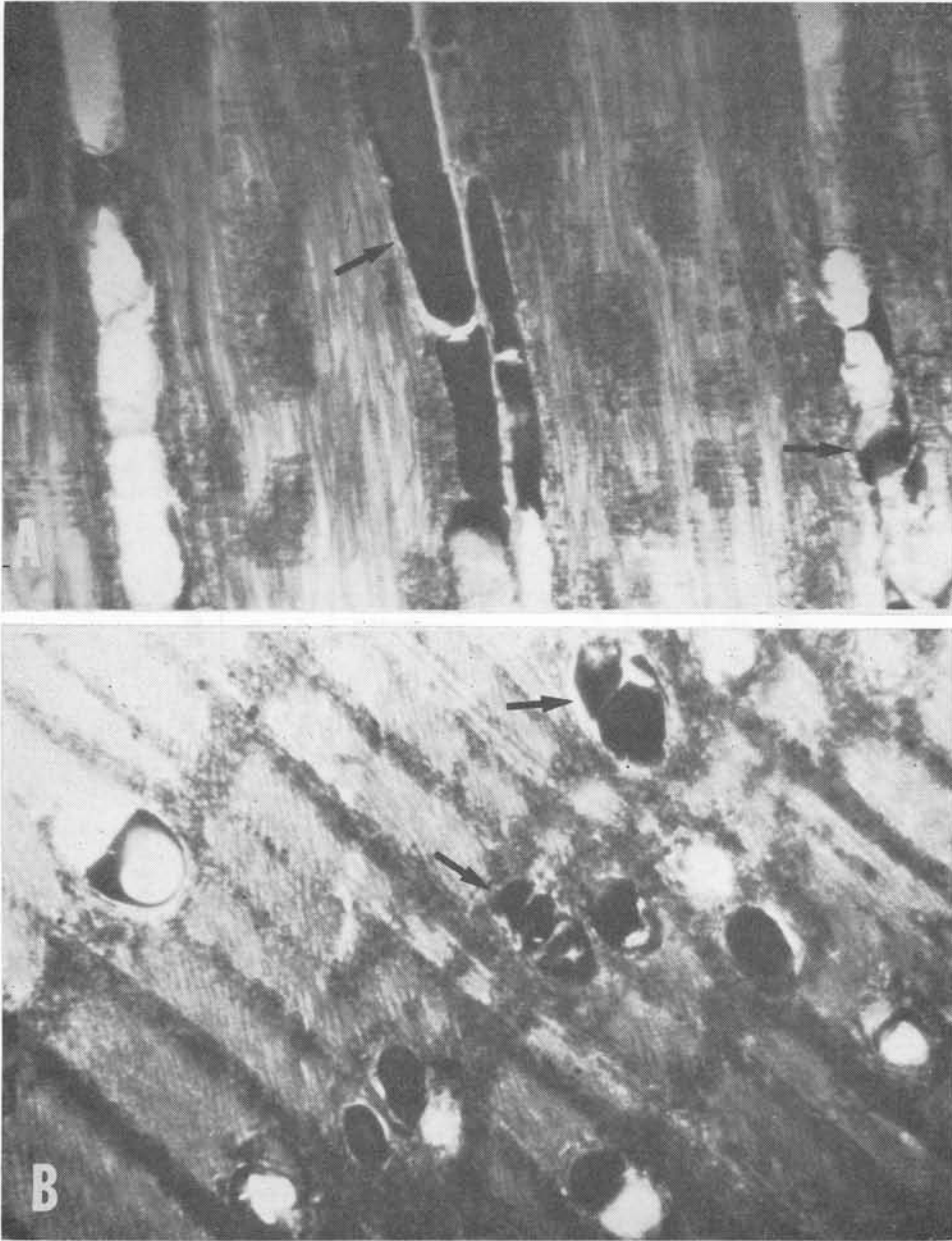


Fig. 2. Vessel plugs from A) longitudinal, and B) cross-sectional sections of decline affected trunk wood, 120X.

present in Brazil. Its presence in apparently healthy trees, however, suggests that tristeza is not involved in the development of the decline syndrome.

Association with nematodes and fungi. Four nematode species: *Tylenchulus semipenetrans* Cobb, *Rotylenchulus reniformis* Linford

and Oliveira, *Helicotylenchus dihystera* (Cobb) Sher and *Criconemoides* sp. were isolated from soil and root samples from healthy and declining trees. Of these species, only *T. semipenetrans* had been proven pathogenic to citrus, inducing symptoms similar to CD. However, none of the nematodes

TABLE 2
HYDRAULIC CONDUCTIVITY (ML/CM²/MIN) OF WOOD PEGS, 0.9 CM IN
DIAMETER x 2.0 CM LONG, OF DIFFERENT PARTS OF HEALTHY AND DE-
CLINING TREES; AVERAGE OF THREE READINGS

Tree condition†	Trunk															
	Main roots		Below budunion			At budunion			Above budunion					Scaffold branches		
	Center	5 mm from cambium	Center	Intermediate	5 mm from cambium	Center	Intermediate	5 mm from cambium	20 cm		60 cm			Center	5 mm from cambium	
Healthy	82.8 a†	72.7 a	0	49.5 a	44.8 a	—	27.9 a	24.6 a	0	30.0	55.2 a	0	45.7 a	52.2 a	18.0 a	64.0 a
CDB-T	43.7 b	18.8 b	—	13.5 b	3.2 b	4.0	15.8 b	10.7 b	0	0	2.1 b	1.4	8.5 b	1.4 b	20.6 a	20.6 b
CDB-A	51.6 c	31.6 c	0	2.9 c	2.7 b	0	2.6 c	3.2 c	0	0	0	0	0	0	0	0.1 c

† Water uptake and zinc level of sampled trees were: healthy, 254 ml and 5.3 ppm; CDB-T, 0 ml and 14.0 ppm; CDB-A, 12 ml and 4.3 ppm, respectively.

‡ Numbers followed by different letters differ significantly at the 5% level.

TABLE 3
 PERCENTAGE OF PLUGGED VESSELS OBSERVED IN DIFFERENT PARTS
 OF WOOD TISSUES OF HEALTHY AND DECLINING TREES†

Tree condition	Trunk										
	Secondary	Main		Below budunion			20 cm above budunion			Scaffold branches	
		Center	5 mm from cambium	Center	Inter-mediate	5 mm from cambium	Center	Inter-mediate	5 mm from cambium	Center	5 mm from cambium
Healthy	3.9 a§	4.8 a	—	10.3 a	0.2 a	0.2 a	32.6 a	26.0 a	21.1 a	5.8 a	0.1 a
CDB-T	37.8 b	7.4 a	18.1	14.1 b	16.7 b	21.3 b	34.8 a	43.4 b	45.1 b	21.4 b	10.7 b
CDB-A	45.9 c	12.4 b	—	16.1 b	21.6 c	15.1 c	36.9 a	37.3 c	34.0 c	—	20.9 c

† Avg. from 12 sections, counting the total number of xylem vessels and the total number of plugged vessels present in a microscope field of approximately 1.8 mm². Counts were made at the center and 2 peripheral areas of each section.

‡ Water uptake and zinc level of sample trees were: 254 ml and 5.3 ppm; CDB-T, 0 ml and 14.0 ppm; and CDB-A, 12 ml and 4.3 ppm, respectively.

§ Numbers followed by different letters differ significantly at the 5% level.

could be associated with either CDB-T or CDB-A. The population (0-32/g sample) was not believed to be large enough to cause decline of 7- to 8-year-old trees. Furthermore, healthy trees had higher nematode populations than the diseased trees.

Fungi, frequently isolated from declínio-affected trees were *Fusarium solani*, Appel & Wr. *Diaporthe citri*, (Fauc.) Wall, *Diplodia natalensis* P. Evans, *Colletotrichum gloeosporioides* Penz and *Botrytis cinerea* Pers. ex Fr. Their possible influence to declínio development has not been tested.

CDB symptom reversal tests. Some studies on blight and blight-like diseases (9, 13, 17, 18) claim to have controlled or improved conditions of the treated trees. Some of these measures have been tested to decrease the damage caused by declínio in the state and in the

country as a whole. Our preliminary results, unfortunately, were unsatisfactory. Growth regulators (either singly or in combination); organic and inorganic fertilizers, and humus applications did not improve growth of the trees. Also, attempts at scion rooting by treating trees with root-promoting hormone (Rootone) 3-5 cm above the budunion and severely pruning them produced normal shoots only temporarily. The scion has produced very few roots up to the present.

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