Comparison of Citrus Tree Declines with Necrosis of Major Roots and Their Association with *Fusarium solani*

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ABSTRACT

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Necrosis of major roots (> 1 cm in diameter) of Dancy tangerine on rough lemon rootstock affected by tangerine collapse (TC) in Florida resembled that on tangerine trees with citrus blight (CB) in Florida and that on navel orange on trifoliate orange rootstock with dry root rot (DRR) in California and that on Eureka lemon on rough lemon rootstock in South Africa. The initial symptom of TC was leaf wilt on trees bearing large crops of fruit; root necrosis was present only on trees that were at least mildly declined and had reduced starch in wood. *Fusarium solani* was isolated from necrotic wood of roots of trees affected with each of these declines. Reduced water uptake and zinc accumulation in trunkwood occurred in trees affected by CB but not in trees affected by DRR and TC. Golden plugs in the xylem of trees affected by DRR and TC resembled those of CB when observed by light microscopy but were filamentous compared with the solid, amorphous plugs of CB when observed by scanning electron microscopy. Inoculation of trifoliate orange seedlings with *F. solani* only caused root necrosis when starch reserves in the wood were first reduced by decapitation and root girdling. *Fusarium* appears to be a primary colonizer of citrus roots after starch reserves are depleted.

Citrus blight (CB), dry root rot (DRR), and tangerine collapse (TC) are tree declines of unknown cause characterized by wilt and dieback of the canopy. CB, which was recently experimentally transmitted (17), is probably infectious. As a tree with CB declines, large roots (> 1 cm in diameter) die (8). Root symptoms resemble those of DRR-affected trees in California (3), and *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. is frequently isolated from necrotic roots (3,8). DRR has commonly been attributed to injuries of the crown or large roots that provide entry wounds for *F. solani* during cool and wet seasons (3,18). TC occurs on cultivars Murcott and Dancy when they have large crops of fruit (15). Depletion of starch reserves in roots as a result of overbearing has been suggested as the primary cause of TC (15), but the condition of the root system in relation to canopy decline has not been evaluated.

Because the symptoms of these diseases are nonspecific, visual diagnosis is difficult. Tests have been used to identify CB and to distinguish it from declines caused by *Phytophthora*, viruses, nematodes, and other agents (1,4). Trees with CB, injected with water by syringe into the trunkwood, take up less water than healthy trees (11). This is associated with amorphous plugs and blockage of water flow in the xylem (5,7). CB-affected trees also characteristically accumulate zinc in the outer trunkwood (19).

In a preliminary survey of CB in South Africa (10), DRR was readily differentiated from CB by these diagnostic tests. Lima and Vasconcellos (12) found no differences in diagnostic characteristics of *declinio* (CB) and TC in Brazil. They concluded that the diseases were similar and suggested that overbearing may influence the development of CB.

The relationship between CB, DRR, and TC needs to be clarified. This led us to compare more extensively the diagnostic characteristics of DRR in California and South Africa with those of TC and CB in Florida, including characterization of plugs in the xylem. Furthermore, because the necrosis of roots is similar for CB and DRR, the role of *F. solani* in these declines and in TC was examined.

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MATERIALS AND METHODS

Tree selection. Citrus trees examined were 15–30 yr old and apparently free of diseases other than those under study. Trees of Dancy tangerine on rough lemon rootstock with CB and TC were located in groves in the ridge area of central Florida (Table 1). Trees of navel orange on trifoliate orange rootstock with DRR were located in two groves in the Sacramento Valley of Northern California (Orland), and trees of Eureka lemon on rough lemon rootstock were located in Eastern Cape Province of South Africa (Sundays River Valley). Diseased trees were selected on the basis of the presence of a necrotic root subtending the root crown after excavation. Necrotic roots were absent on apparently healthy trees. Canopy symptoms were rated on the basis of a modification of a rating system used for blight (8), where 0 = healthy, 1 = mild (slight wilt but little or no thinning of foliage), 2 = moderate (leaves flaccid, canopy sparse with some dieback), and 3 = severe (canopy thin, twig dieback substantial). Trees with symptoms transitional between the categories were rated 0.5, 1.5, and 2.5.

Diagnosis. Trees were tested for water conductivity in the trunk by the syringe injection method (11) and for zinc accumulation in the trunkwood (19). For starch analysis, part of the wood sample collected above the bud union for zinc analysis was used. A wood sample below the bud union was taken from the root crown proximal to a root with necrosis. Duplicate 1-g samples of wood ground to pass through a 0.42-mm mesh screen were digested in perchloric acid, and starch content was estimated colorimetrically by the iodide-iodate method (6).

A horizontal core sample of the trunk was taken with a 5-mm increment borer just above the hole made for the syringe injection test. Cores were fixed and sectioned as described previously (4). Transverse sections, 30–40 μm thick from the portion of the core 2–3 cm from the cambium, were mounted and the brown and golden plugs were counted in 200 xylem vessels in random fields at $\times$100 by transmitted-light microscopy. The remaining portions were prepared for scanning electron microscopy (SEM) as described previously (4).

Root necrosis evaluation. At Lake Alfred, FL, 12 apparently healthy Dancy tangerine trees and trees in every decline category were lifted with a tree puller to expose the entire root system. At least four trees in each category were observed (Fig. 1). Root necrosis on each tree was rated by observing 10 major roots extending from the trunk plus their distal roots down to 1 cm in diameter (8).

Fungi were isolated from discolored tissue and healthy-appearing tissue at the margin in roots 1–10 cm in diameter as described previously (8). Single-spore cultures of all fungal isolates identified on Bermuda hay agar (16) as *F. solani* were maintained on V-8 juice agar (13).

Inoculations. A modification of the inoculation technique of Bender and Menge (2) was used to study the relationship between starch reserves and *Fusarium* infection of trifoliate orange roots. Before inoculation, five seedlings with trunks 2–3 cm in diameter were decapitated 5–10 cm above the root crown and allowed to resprout. Another five seedlings were left intact. Three roots greater than 0.5 cm in diameter on each seedling were treated as follows: One was girdled by removal of a 4-mm strip of cambium, another was girdled and inoculated with a piece of rough lemon root colonized with *F. solani* (isolated from a blighted tree in Florida [8]) that was inserted into a drill hole 1 cm below the girdle, and the third was girdled and inoculated with an autoclaved piece of *F. solani*-colonized root. Inoculation holes were wrapped with plastic grafting tape. Trees were maintained in a screenhouse and examined 1 mo after inoculation. At this time, wood samples were taken from the stem for starch analysis and from the root above and below the inoculation point for evaluation of xylem plugging. The inoculation procedure was performed twice during the summer of 1984 with similar results.

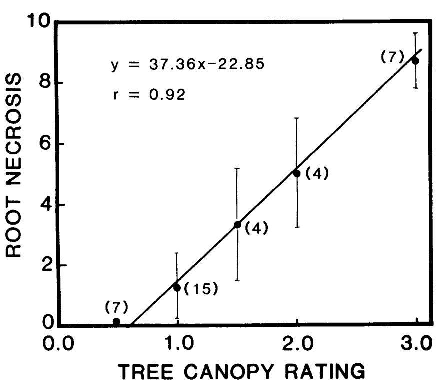

Fig. 1. Relationship of root necrosis to canopy symptoms in Dancy tangerine on rough lemon rootstock affected by tangerine collapse at Lake Alfred, FL. Number of trees of each canopy condition indicated in parentheses. Linear correlation of canopy rating (1 = mild, 2 = moderate, and 3 = severe wilt and dieback) with root necrosis (number of major roots with lesions on distal roots down to 1 cm in diameter) significant at $P<0.01$.

RESULTS

Symptoms and diagnostic characteristics. Root necrosis on Dancy tangerine trees affected by TC or CB resembled that previously reported on sweet orange trees with CB in Florida (8) and DRR in California (3). Visual symptoms of tangerine trees with TC differed from those of CB in that only trees with excessive crops of fruit were affected and the canopy was sectored, i.e., early symptoms were confined to one or two scaffold branches and one major subtending root. Unlike CB and DRR, where the necrosis was usually below the bud union, necrosis of wood and bark of trees with TC extended above the bud union into the trunk and large limbs. Sporodochia of *F. solani* were visible on dried necrotic bark overlying the affected wood. Isolations from discolored wood in roots always yielded *F. solani*, whereas this fungus was absent from adjacent healthy-appearing tissue. No other fungi or bacteria were consistently isolated from discolored wood in roots.

Although root necrosis was common to all three tree declines, elevated zinc levels in wood and decrease in water uptake were observed only in those tangerine trees at Windermere, FL, that were diagnosed as having CB (Table 1). The significantly less water uptake into the trunk xylem was associated with the presence of plugs that completely filled the vessel lumens and appeared golden by light microscopy (Table 1). Golden plugs were also observed in tangerine trees with TC at Avon Park, FL, and trees with DRR in California but not in trees with DRR in South Africa. The presence of golden plugs in TC and DRR was not associated with significant reduction in water uptake even though the number in TC-affected trees was comparable to that in CB-affected trees.

Although golden plugs in DRR and TC resembled those in CB-affected trees under light microscopy, these plugs were filamentous in structure under SEM (Fig. 2C–F). The plugs appeared porous compared with the solid, amorphous appearance of plugs found in CB-affected trees (Fig. 2A [inset], C–F). Significantly more brown plugs were found in CB-affected trees than in healthy trees or those affected by the other declines (Table 1). Brown plugs in CB, DRR, and TC were filamentous in structure (Fig. 2B). The filamentous plugs, regardless of color and the type of tree decline, appeared to vary greatly in size and structure of the filaments (Fig. 2B[inset],D,F).

Trees affected by each of the three declines had significantly lower starch levels in the wood than did healthy trees, except for samples above the bud union of DRR-affected navel orange on trifoliate orange rootstock (Table 1). Starch levels above and below the bud union did not differ significantly ($P<0.05$) for diseased or healthy trees of any of the scion/stock combinations, indicating that a dysfunction of the bud union was apparently not the cause of the declines. There was no visible gumming or necrosis in the cambium at the bud union. Overall, starch levels in healthy navel orange on trifoliate orange rootstock were substantially lower than

Table 1. Characteristics of healthy (H) and declining citrus trees with citrus blight (CB), dry root rot (DRR), and tangerine collapse (TC) in Florida, California, and South Africa

Location and scion/rootstock[a]	No. of trees	Canopy rating[b] (0–3)	Zinc (μg/g)	Water uptake (ml/sec)	No. of plugs/ 200 vessels		Starch (mg/g)		Tree diagnosis
					Golden	Brown	Above bud union	Below bud union	
Florida									
(Windermere)	6	0.0	1.4	0.20	0.0	0.0	37.3	42.5	H
DT/RL	6	1.2	3.4*[c]	0.03*	13.5*	6.7*	9.6*	23.9*	CB
Florida									
(Avon Park)	6	0.0	1.3	0.15	0.0	0.5	30.4	49.8	H
DT/RL	6	1.5	2.1	0.08	12.2*	2.7	14.7*	26.5*	TC
California	12	0.0	1.0	0.34	0.4	1.2	6.7	6.2	H
NO/TO	12	1.9	0.8	0.30	2.3	6.7	5.9	3.9*	DRR
South Africa	10	0.0	2.2	1.6	0.0	0.0	31.3	40.4	H
EL/RL	10	1.5	1.9	1.9	0.0	0.0	14.3*	25.3*	DRR

[a] DT = Dancy tangerine (*Citrus reticulata* Blanco), RL = rough lemon (*C. jambhiri* Lush), NO = navel orange (*C. sinensis* (L.) Osbeck), TO = trifoliate orange (*Poncirus trifoliata* (L.) Raf.), and EL = Eureka lemon (*C. limon* (L.) Burm. f.).

[b] Canopy rating: 0 = healthy, 1 = mild, 2 = moderate, and 3 = severe wilt and dieback.

[c] Means significantly different from H controls according to Student's *t* test at $P = 0.05$.

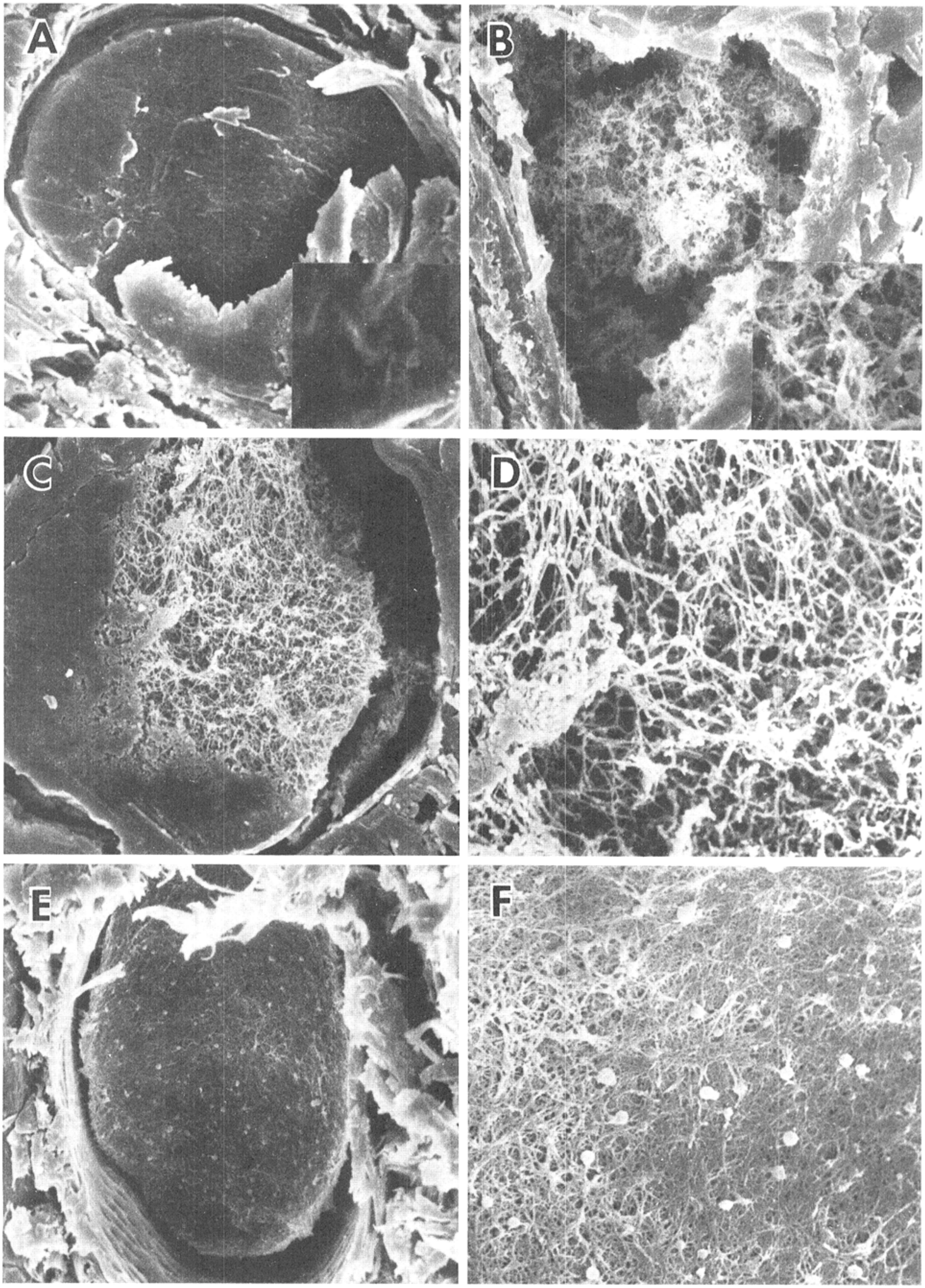

Fig. 2. Scanning electron microscopy (SEM) of plugging material, which appears golden or brown under light microscopy, in xylem of declining citrus trees. **(A)** Golden plug in xylem of a blight-affected Dancy tangerine tree appears solid (×1,000). Inset shows higher magnification of the same plug (×3,000). **(B)** Brown plug in xylem of blight-affected tangerine tree appears filamentous (×1,000). Inset shows higher magnification of the same plug (×3,000). **(C)** Golden plug in dry root rot-affected navel orange tree appears porous under SEM (×1,000). **(D)** Higher magnification of C reveals the filamentous structure of plug (×3,000). **(E)** Golden plug in Dancy tangerine collapse-affected tree appears porous and similar to that in dry root rot (C and D) (×1,000). **(F)** Higher magnification of E reveals filamentous structure of plug (×3,000).

in declining trees of other scion/stock combinations at other locations.

Relationship between canopy symptoms and root necrosis. There was a highly significant linear correlation between canopy symptoms and number of large necrotic roots on TC-affected trees at Lake Alfred (Fig. 1). The absence of root necrosis on trees with incipient leaf wilt (0.5 canopy rating) was evidence that root damage followed rather than preceded canopy damage.

Inoculations. Starch levels in wood of intact trifoliate orange seedlings were significantly ($P < 0.01$) higher than in decapitated plants (7.4 vs. 1.2 mg/g). None of the roots on intact trees was affected by inoculation. However, two of the five decapitated seedlings developed necrosis of bark and wood below the girdle on the root inoculated with *F. solani*. In one case, necrosis extended across the girdle on the underside of the root and up the side of the stem. *F. solani* was isolated from wood at the margin of necrosis in the stem but was not present in healthy-appearing tissue. Unaffected xylem in the root above the necrosis contained plugs similar to those observed by SEM in DRR and TC.

DISCUSSION

The association of *F. solani* with necrosis of major roots on Dancy tangerine on rough lemon rootstock with TC was similar to that on CB-affected tangerines and oranges (8) in Florida and DRR-affected oranges in California (3) and lemons in South Africa (10). Root necrosis has also been observed on collapsing Murcott tangerine on rough lemon rootstock in Florida (J. H. Graham, *unpublished*). In these tangerine varieties, TC occurred in trees bearing large crops, unlike the trees affected by CB that had normal crops of fruit. Decline in the canopy of TC-affected trees began in one or two scaffold limbs, and early symptoms of wilt appeared before root necrosis. Subsequent decline of the canopy was associated with an increase in the number of roots with necrosis, as observed for declining orange trees affected by CB (8).

Infection by *F. solani* occurred after there was a stress on the tree, i.e., overbearing in tangerines or xylem dysfunction in CB-affected trees. These stresses resulted in significant depletion of starch reserves even in mildly declined trees. We found that *F. solani* would not colonize a trifoliate orange root unless starch reserves were depleted by removal of photosynthetic tissues and girdling of the roots above the point of inoculation. Thus, as we concluded previously (8), *F. solani* appears to be a primary colonizer of citrus roots after depletion of starch reserves in the wood.

The stress factors that predispose to DRR are not fully known. Bender et al (2,3) concluded, as we have, that reduction in growth and vigor of roots is necessary before *F. solani* can infect citrus wood. Poor drainage is associated with increased incidence of DRR in California (18). In California and South Africa, groves were watered by flood irrigation. This factor, in conjunction with the inherently low starch reserves in navel oranges on trifoliate orange rootstock in California and high populations of citrus nematode in South Africa (L. J. Marais, *unpublished*), may have predisposed roots to infection by *Fusarium*. Hamid et al (9) recently demonstrated that repeated root initiation in response to infection by citrus nematode results in starch depletion in fibrous roots.

Colonization of roots by *F. solani* was not associated with the development of amorphous plugging in the xylem or zinc accumulation in the trunkwood of DRR or TC trees. Plugs in xylem of DRR- and TC-affected trees only superficially resembled amorphous plugs in CB. Golden plugs in DRR and TC were filamentous in structure and did not significantly reduce water conductivity in the trunk.

In TC-affected trees, water flow was reduced somewhat in unaffected trunkwood in proximity to necrotic tissue. We observed the formation of plugs, similar to plugs in DRR and TC, adjacent to necrotic tissue in trifoliate orange seedlings. Nemec et al (14) and Bender et al (3) suggested that plugs are formed in response to the presence of *F. solani* and the production of toxins. Nemec et al (14) concluded that *F. solani* is a primary pathogen of citrus roots and the primary incitant of the plugging in blight-affected trees. We believe that *F. solani* is not the primary cause of the declines studied and that infection of citrus roots by *F. solani* does not result in formation of amorphous plugs typical of those in CB-affected trees.

DRR and TC can be controlled through modification of cultural practices to reduce primary stresses. With tangerines, fruit thinning can reduce the incidence of TC (15). In California, maintenance of good soil drainage and careful irrigation have been suggested as practices for preventing DRR (18).

Necrosis on the roots cannot be used as a diagnostic character of CB because other declines have similar root symptoms. In fact, it is difficult to distinguish tangerine trees with CB from those with TC without conducting both the zinc and water uptake tests. Nevertheless, these diseases are separable by these tests and are not of a similar nature as previously implied (12).

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