

## Necrosis of Major Roots in Relation to Citrus Blight

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### ABSTRACT

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Necrosis of bark and wood of major roots (>1 cm diam.) was observed on sweet orange (*Citrus sinensis*) trees on rough lemon (*C. jambhiri*) rootstock affected by citrus blight. The number of lesioned roots increased as the tree canopy declined and as diagnostic symptoms of blight, zinc accumulation, and restricted water flow in the trunk wood developed. *Fusarium solani* was consistently isolated from necrotic roots but did not cause lesions when roots of rough lemon seedlings or field trees on rough lemon rootstock were inoculated. Roots of blighted trees may have been susceptible to infection by *Fusarium* as a result of xylem blockage in the trunk, canopy decline, and subsequent decrease in starch reserves in the tree.

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Citrus blight is characterized by xylem dysfunction, which results in restricted water flow in the trunk (4,5), and by zinc accumulation in the trunk wood (19). These symptoms are apparently specific for blight, which is difficult to diagnose on the basis of canopy symptoms alone

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(13). Blight is most devastating to trees on rough lemon (*Citrus jambhiri* Lush.) rootstock but affects trees on most rootstocks commonly used in Florida (21). The cause of citrus blight is unknown although xylem-limited bacteria transmitted by sharpshooter vectors (8) and feeder-root-inhabiting *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. (10) have been suggested as possible causal agents.

Root systems of blighted trees have been reported to appear healthy, at least in the early stages of the decline (3,7,13). Blight has been attributed to feeder-root loss caused by *F. solani* (10), but there are no reports of constant association of necrosis of major roots (>1 cm diam.) with the disease. Some early descriptions of blight (6,14) refer to necrosis that begins in smaller roots and progresses

into larger ones. *Fusarium*, *Diplodia*, and *Phoma* were reportedly isolated from lesioned roots (6). These observations were made before diagnostic tests for blight were available (4,19); therefore, it is uncertain whether the tree declines studied in those cases were caused by the same disease. More recently, Young and Garnsey (22) observed necrosis of large roots of known blight-affected trees on rough lemon and Carrizo citrange (*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck) rootstocks.

The purpose of this study was to determine the relationship of necrosis of major roots with development of decline in the tree canopy and other diagnostic symptoms of blight. Isolations were made from lesioned roots and pathogenicity of the fungal isolates was tested.

### MATERIALS AND METHODS

**Tree selection.** Citrus trees surveyed were Pineapple, Hamlin, and Valencia sweet orange cultivars (*C. sinensis*) on rough lemon rootstock. All trees were at least 18 yr old and were located in eight groves, four in the ridge area of central Florida (Lake Alfred, Auburndale, Haines City, and Lake Garfield) and four in the flatwoods area of southern (Ona) and southeastern Florida (Vero Beach and Indiantown, two groves). In the Ona grove, where freeze injury occurred in January 1981 and 1982, freeze-damaged

trees not affected by blight but with severe canopy loss were selected for comparison with root systems of blight-affected trees.

Healthy trees and those affected by blight were rated based by visual appearance of the canopy on a scale of 0-3, where 0 = healthy, 1 = mild (leaves small with symptoms of zinc deficiency, internodes short, slight wilt but little or no thinning of foliage), 2 = moderate (leaves small, often flaccid, with symptoms of zinc deficiency, canopy sparse with some twig dieback), and 3 = severe (canopy thin, twig dieback substantial, trunk sprouts common). Trees with symptoms transitional between categories were rated 1.5 or 2.5.

Trees in every blight category were selected from each grove site for analysis. Of 168 trees surveyed, the number analyzed in each category was: 38 healthy trees, 14 in category 1, 13 in category 1.5, 31 in category 2, 31 in category 2.5, and 41 in category 3. Although it was not possible to perform blight diagnoses and root evaluation on all trees in some groves, at least 12 trees were analyzed for blight and root necrosis in each category and used for correlation with tree canopy rating.

**Blight diagnosis.** Water uptake into the trunk was measured by the gravity-infusion method of Cohen (4). Holes 8 × 40 mm were drilled in the trunk above the bud union and metal injectors were tapped 1 cm into the hole. Water uptake through the injector from a calibrated bottle was measured for a 24-hr period.

For each tree, samples of trunk wood about 20 cm above the bud union were collected by drilling holes 2.5 cm deep with a 1.27-cm-wide wood drill bit. Duplicate 1-g samples were oven-dried and zinc concentration in wood was determined (19). Starch content of 1 g of finely ground wood from the same trunk sample was determined by perchloric acid digestion and colorimetric determination by the iodide-iodate method (2).

**Root necrosis evaluation.** After blight diagnoses were performed, trees were lifted with a tree-puller to expose the entire root system. In the process of lifting trees, some of the smaller roots were broken off, especially from root systems of healthy trees. Root necrosis on each tree was rated by observing 10 intact major roots extending from the trunk plus their distal roots down to 1 cm in diameter. The rating was expressed as the number of roots with necrotic bark and wood (lesions) per tree.

**Isolation from necrotic roots.** For four groves, isolations from xylem in roots that ranged from 1 to 10 cm diameter were made at the margin between discolored and healthy tissue. For this, roots were surface-disinfested in 10% commercial chlorine bleach for 10 min, rinsed with sterile water, and split open aseptically. Pieces of wood were plated on Komada's medium (9) and potato-dextrose agar (Difco) (PDA). All fungal isolates were identified on Bermuda hay agar (16) as *F. solani*, which was single-spored and maintained on V-8 juice agar (17).

**Inoculation studies.** Pathogenicity of *F. solani* was evaluated by inoculating rough lemon seedlings with one isolate from necrotic roots of a tree affected by blight and one isolate from a tree affected by dry root rot in California (1). Each isolate was grown in an autoclaved mixture of 1,000 ml vermiculite and 350 ml V-8 juice medium. After 4 wk, inoculum was harvested and samples plated on PDA to ensure that vermiculite was uniformly infested and not contaminated. Roots of 9-mo-old rough lemon seedlings were wounded by severing the taproot, and 100 cm<sup>3</sup> of vermiculite inoculum was packed around the wounded portion of the root system as it was transplanted into autoclaved (6 hr at 121 C) Candler fine sand soil (pH 6.8) in 2-L pots. Control plants were inoculated with an autoclaved mixture of inoculum of both isolates. Eight plants per treatment were placed in each of three circulating water tanks maintained at 15, 20, and 25 C. Plants were fertilized after 1 mo with Hoagland's solution and harvested 2 mo after inoculation.

For inoculation of 2-yr-old rough lemon seedlings (trunk 1.5 cm diam.) in the greenhouse, two isolates of *F. solani* from necrotic roots were grown on segments of autoclaved rough lemon roots (0.5 × 1.5 cm) embedded in 1.5% water agar. Uniformly colonized root pieces were inserted into a hole drilled in the crown of the root system either 1 cm above or below the soil line. Controls received autoclaved inoculum pieces of each isolate. Inoculation holes were sealed with petroleum jelly and those below soil line were re-covered with soil. Nine trees per treatment were inoculated in January 1982 and examined after 6 mo.

To ascertain whether *F. solani* causes lesions in roots in the field, 8-yr-old Pineapple sweet orange trees on rough lemon rootstock were inoculated with root pieces as described before. Large roots were exposed by washing soil from around trees and at least two *Fusarium* and two control inoculations per root system were performed before recovering the roots with soil. Inoculation points on the same root were at least 2 cm apart. Two replicate trees were inoculated in January 1982 and two in March 1982, and roots were examined in August 1982.

## RESULTS

Necrotic roots were always associated with canopy symptoms but were observed on only two of 12 healthy trees examined. The number of necrotic roots was highly correlated ( $r = 0.97$ ) with severity of canopy decline (Fig. 1A). Diagnoses by water uptake and zinc analyses of trunk wood confirmed that visual symptoms were those of blight. Canopy damage was linearly correlated ( $r = 0.92$ ) with zinc content in trunk wood and negatively logarithmically correlated ( $r = -0.99$ ) with water uptake into trunk

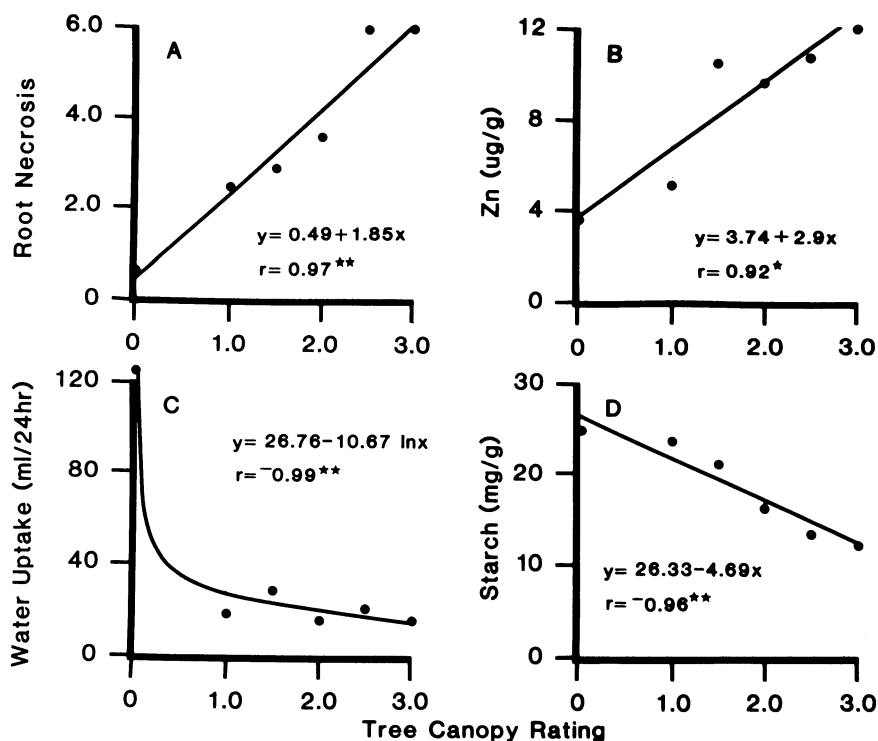


Fig. 1. Development of root necrosis and diagnostic symptoms of blight on sweet orange/rough lemon (scion/stock) trees in relation to visual symptoms. Correlation of canopy rating (0 = healthy, 1 = mild, 2 = moderate, and 3 = severe) with (A) root necrosis rating (number of major roots with lesions on distal roots larger than 1 cm diam.), (B) zinc content of trunk wood, (C) water uptake into trunk, and (D) starch content of trunk wood. Correlations significant at  $P = 0.05$  (\*) and 0.01 (\*\*).

wood (Fig. 1B,C). Canopy loss was also associated with decrease ( $r = -0.96$ ) in starch content of trunk wood, which is indicative of the weakened condition of trees affected by blight (Fig. 1D).

In two sectored trees (those in which the earliest canopy symptoms were confined to one or two major limbs), lesions occurred on at least one major root subtending the symptomatic limbs; necrotic roots were not present on the healthy-appearing side of the tree. Similar observations were made for five

**Table 1.** Canopy damage and the occurrence of root necrosis on blighted and freeze-damaged Valencia orange/rough lemon (scion/stock) trees in June 1982

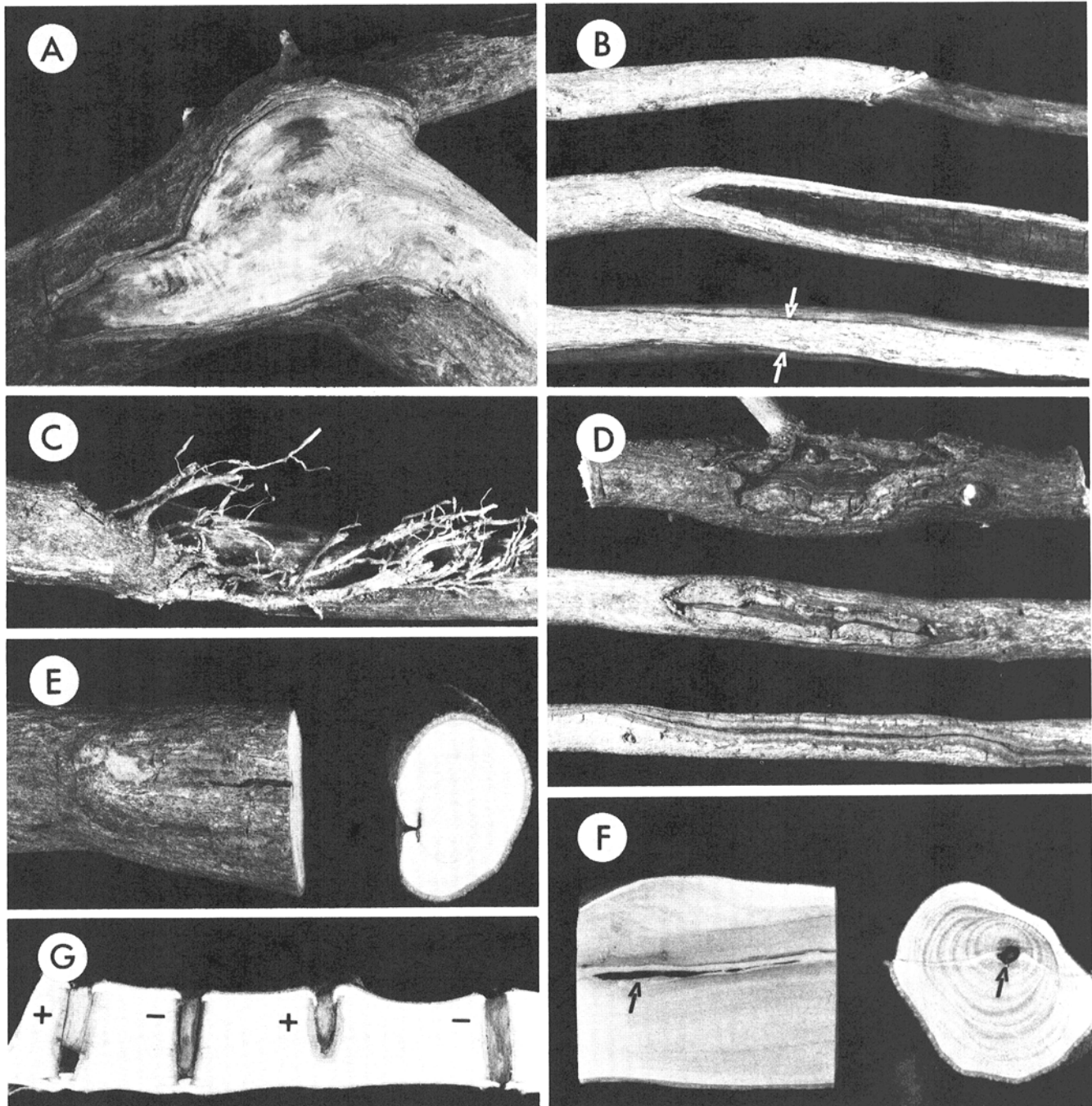
Tree condition	Tree canopy rating <sup>a</sup>	Root necrosis <sup>b</sup>	Water uptake into trunk (ml/24 hr)	Zn content of trunkwood ( $\mu\text{g/g}$ )	Starch content of trunkwood (mg/g)
Freeze-damaged <sup>c</sup>	3 y <sup>d</sup>	0 y	692 y	9.1 y	17.4 y
Blighted	3 y	5 z	16 z	16.5 y	15.5 y

<sup>a</sup>Visual symptoms of blight: 0 = healthy, 1 = mild, 2 = moderate, and 3 = severe.

<sup>b</sup>Number of 10 major roots with lesions present on distal roots larger than 1 cm in diameter.

<sup>c</sup>Trees suffered freeze-damage of the canopy in January 1981 and 1982 comparable to a blight rating of 3.

<sup>d</sup>Column values are means of five observations. Means followed by different letters are significantly different at  $P = 0.05$ .



**Fig. 2.** Necrotic roots of rough lemon rootstock trees from the field. (A) Necrosis of a smaller root subtending a major root; note loss of bark revealing grayish stains in the wood. (B) Discrete margin formed between healthy and necrotic tissue in smaller roots; note strip of live cambium (arrows) on topside of root. (C) Proliferation of adventitious roots from callus tissue formed at margin of necrotic tissue. (D) Wounds on healthy roots caused by root weevils feeding on cambium. (E) Cross section of root weevil lesion revealing necrotic area that is nearly callused over. (F) Longitudinal and cross section of root showing necrotic area (arrows) in wood surrounded by discolored tissue. (G) Longitudinal section of major root revealing no increase in discolored wood around drill hole where *Fusarium solani* was inoculated (+) compared with control (-).

additional sectored trees not removed from the ground but evaluated by removing soil from around the roots in place. Water uptake occurred on the healthy side of the trunk but not on the blighted side, whereas zinc content was high ( $\bar{x} = 7.00 \mu\text{g/g}$  tissue) on both sides of the trunk.

Root necrosis did not occur on trees with canopy damage caused by freezes in January 1981 and 1982 even though starch levels in tissue were comparable to severely blighted trees in this grove (Table 1) and other locations (Fig. 1D). Six months after the freeze, trees with freeze damage could be distinguished from blighted trees not only by the absence of root lesions but also by the water uptake test.

As observed previously (6,14), necrosis appeared to originate in small roots, then progress into larger roots up to 10 cm diameter (Fig. 2A). The appearance of necrotic roots was similar to that of dry root rot in California that has been associated with *F. solani* (1). Initially, roots showed moist, dark decay in the bark, which dried and cracked to reveal brownish gray stain in the wood (Fig. 2A,B,F). Occasionally, the decayed bark and cambium was stained purple or red; gumming was never observed. The zone between healthy and discolored wood was usually discrete, and adventitious roots occasionally proliferated from callus tissue formed at this point (Fig. 2C). Lesions involved both xylem and bark, often developing along one side of the root (usually the underside) (Fig. 2B).

Isolations from discolored wood at the margin yielded *F. solani*, whereas *Fusarium* was absent from adjacent healthy-appearing tissue. No other fungi or bacteria were consistently isolated from discolored wood in roots. *Fusarium* was also isolated from small, necrotic areas in the root resulting from wounds caused by mechanical damage from disking operations or by root weevils feeding on the bark (Fig. 2D,E). Necrotic areas were apparently callused over (Fig. 1E), and on blight trees, they appeared to act as foci from which *F. solani* invaded adjacent root tissue (Fig. 2F). On healthy trees, these wounds rarely developed into root lesions even though *Fusarium* was present in the necrotic tissue.

Inoculations of rough lemon seedlings with isolates of *F. solani* from a root lesion on a blight-affected tree or from a tree affected by dry root rot did not result in measurable differences in root or shoot growth compared with uninoculated controls at any of the three soil temperatures (15, 25, or 30 C). Necrosis was not observed at the site of wounding on the taproot. Inoculation of older seedlings with infected root pieces also did not result in necrosis of adjacent root crown and stem tissue. Results from root inoculations in the field were also negative. A longitudinal section of a

major rough lemon root revealed no increase in discolored wood around the drill hole where *Fusarium* inoculum was inserted compared with the uninoculated control (Fig. 2G).

## DISCUSSION

Necrosis of major roots associated with *F. solani*, resembling Fusarium dry root rot of citrus in California, is present on rough lemon rootstock trees with citrus blight in Florida. *Fusarium* root necrosis is also present on blight trees on Carrizo citrange and trifoliolate orange (*Poncirus trifoliata*) rootstocks (22; J. H. Graham and L. W. Timmer, unpublished) and may occur on all rootstocks affected by blight. The increase in lesioned roots is correlated with development of canopy and diagnostic symptoms of blight. Root necrosis rarely occurs on healthy trees even though wounds are present and *F. solani* is established in the wood tissue.

*Fusarium* isolates tested did not cause lesions on healthy rough lemon roots even when mechanically wounded and grown at low temperatures that might inhibit wound response (12). Our negative observations with isolates of *F. solani* from California and Florida contrast with previous studies (11,18) that reported that at 15 C, *F. solani* decreased top growth and increased loss of fibrous roots due to rot. Our results support those of Bender et al (1), who found that at 15 C, *Fusarium* isolated from dry root rot did not cause stem lesions or root rot on Troyer citrange. When seedlings were preinoculated with *Phytophthora citrophthora* to form a lesion and subsequently inoculated with *F. solani* in the lesion, however, the combined inoculation at low temperature resulted in *Fusarium* infection of stem and root tissue and seedling mortality. Thus, it appears that continual stress factors such as *Phytophthora* plus low temperature or blight are necessary for infection of citrus wood by *F. solani*.

Young and Garnsey (22) found root necrosis on trees with sectored canopies that they considered the earliest visual manifestation of citrus blight. As previously reported (22,23), impaired water uptake and root lesions are associated with the symptomatic side of the tree but not the healthy-appearing side. Zinc accumulation in the wood, which can occur several months before appearance of canopy symptoms (20), is evident on both sides of the tree, indicating the entire tree is diseased (22,23). Thus, root rot apparently is not the cause of blight but is a response to the xylem blockage in the trunk (5) that results in water-stress symptoms in the canopy, reduction in leaf area (15), and depletion of starch reserves in the tree. Root necrosis does not occur after canopy loss and decrease in starch as a result of periodic freeze-damage, which indicates that roots are susceptible to *Fusarium* infection only after a prolonged

decline in starch reserves as observed for blight trees.

## ACKNOWLEDGMENTS

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