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A Survey for Citrus Blight in South Africa*

R. F. Lee, L. J. Marais, and R. H. Brlansky

ABSTRACT. A survey was conducted in the major citrus producing areas of South Africa and Swaziland to verify the presence of citrus blight and to determine the distribution of the disease. Trees on rough lemon rootstock having blight-like symptoms as well as apparently healthy trees in each area were tested to diagnose for blight using water injection tests, zinc analysis of trunk xylem, and, in some cases, cytological examination for plugs in the xylem vessels. Based on these tests, blight occurs in South Africa in the Northern Transvaal, Eastern Transvaal, Natal, and Eastern Cape Province areas and in Swaziland. The incidence of blight was greater in the warmer areas of the Transvaal.
Index words. stem pitting, root necrosis.

Citrus blight, the most devasting decline disease of citrus in Florida, was first reported in Florida over 100 years ago (11). Recently citrus blight has been reported in other countries (3, 6). In South Africa, a decline resembling citrus blight was first noticed in the Transvaal in the 1970's and subsequently reported (3, 7). One purpose of this study was to confirm the presence of citrus blight in South Africa using the diagnostic tests currently used in Florida [comparative symptomatology (11), accumulation of zinc in trunk wood (13), reduced water uptake (4, 9), and the occurrence of amorphous plugs in trunk xylem of blight-affected trees (5)]. Another objective was the distribution of the disease in the citrus-producing areas of South Africa and Swaziland.

MATERIALS AND METHODS

Citrus trees on rough lemon rootstock with blight-like decline symptoms (11) were selected in the major citrus producing areas of South Africa and in Swaziland. Groves 1 through 7 were in the Northern Transvaal, 8 through 13 were in the Eastern Transvaal, 14 through 16 were in Natal, 17 through 20 were in the Sundays River Valley (SRV) of Eastern Cape Province, and Groves 21 and 22 were in Swaziland. Individual trees were rated visually on a scale of 0 = healthy; 1 = mild (small leaves with zinc deficiency symptoms, short internodes, slight wilt but little or no thinning of foliage); 2 = moderate (small leaves, often flaccid, with zinc deficiency symptoms, sparse canopy with some twig dieback); 3 = severe (thin canopy, substantial twig dieback, and trunk sprouts common). In some areas, especially in the cooler areas of Eastern Transvaal and Natal, greening disease was widespread. The mottled leaf pattern and yellowing along the leaf midrib is unique to greening (12), thus it was possible to select a number of apparently healthy trees and an equal number of trees having typical blight-like symptoms without greening symptoms in each grove to be tested. The age of the trees tested ranged from 7 to 25 years with the average about 15 years. Trunk wood was collected from two sides of the tree using a zinc-free drill bit. Wood samples from each tree were combined,

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oven-dried, and analyzed for zinc content as previously described (13, 14).

Water uptake was determined using the syringe injection method of Lee *et al.* (9) and in some groves by the gravity infusion method of Cohen (4). A 4-mm hole 30-mm deep was drilled into the tree trunk for the syringe injection method. The time needed to inject 10 ml water into the trunk using a 30 ml eccentric luer-tip syringe was recorded. Results for the syringe injection method are expressed as ml of water injected per second. For the gravity infusion method, the plastic enema bags described by McCoy (10) for injecting tetracycline into palm trees were used. Results are expressed as ml of water taken up by the tree per 24 hours.

Core samples were obtained about 25-30 cm above the budunion from healthy and declining trees in several locations using a Haglof 5 mm Swedish increment borer. Cores were fixed in 3% glutaraldehyde in 0.066 M sodium phosphate buffer pH 7.0 for 48 hours, washed, and stored in 0.05 M sodium phosphate buffer, pH 7.4, 0.15 M sodium chloride and 0.02% sodium azide (PBS buffer). Cross sections 40-50 μm thick were cut from the core at a depth of 2 cm from the cambium with a cryostat. Ten sections from each core were mounted on slides and viewed unstained under a light microscope. For scanning electron microscopy (SEM), a 2-3 mm long piece from the same area of the core was dehydrated in a 30-100% acetone series (20 minutes for each step). The specimens were then transferred to an acetone: freon (2:1, 1:2) series for 20 minutes each and then into two changes of 100% freon and finally critical point dried in a Bomar Critical Point Drier (The Bomar Co., Tacoma, WA 98401). The specimens were cut for longitudinal or transverse viewing, mounted on SEM stubs, sputter coated with 100 Å gold palladium and viewed in a JEOL JSM35 scanning electron microscope.

A bark patch (~0.5 x 2.0 cm) was taken across the budunion from each tree. The bark patch was fixed in glutaraldehyde and washed in PBS buffer as described for core samples. The bark patch and xylem under the bark patch were examined for stem pitting. Stem pitting on the rootstock was rated on a scale of 0 to 3 where 0 = no stem pitting; 1 = mild stem pitting; 2 = moderate; and 3 = severe stem pitting.

Previous work by Graham *et al.* (8) with blight in Florida and Bender *et al.* (2) with dry root rot in California has shown that declining citrus trees may have extensive root necrosis. Root systems were exposed by pulling trees or by digging and then washed free of soil using water under pressure. The presence and extent of root necrosis on healthy and declining trees at several locations was then evaluated.

RESULTS

The following criteria were used to determine if the tree decline was due to citrus blight: characteristic foliar symptoms of blight (11), accumulation of zinc in the trunk xylem (13), reduced water uptake (4, 9), and the presence of amorphous plugs in trunk xylem vessels (5). Table 1 summarizes the visual ratings, zinc levels, and water uptake data by grove of the trees tested. All of the groves tested in the Northern and Eastern Transvaal (Groves 1 through 12) and in Swaziland (Groves 21 and 22) had blight. In Natal, only one grove of the three tested had blight (Grove 14). While Groves 15 and 16 had foliar symp-

TABLE 1
SUMMARY OF VISUAL RATINGS, ZINC ACCUMULATION IN TRUNK WOOD, AND WATER UPTAKE IN DECLINED AND HEALTHY TREES TESTED FOR THE PRESENCE OF CITRUS BLIGHT IN DIFFERENT AREAS OF SOUTH AFRICA

					Water uptake		
Grove no.	Scion/stock†	No. of trees	Visual rating‡	Zinc (µg/g)	syringe (ml/sec)	bottle (ml/day)	Diagnosis§
Northern Transvaal							
1	Val/RL	4	0	0.94	2.00	—	—
		4	1.8	16.31*	0.00*	—	Blight
2	Val/RL	10	0	1.38	1.54	43.5	—
		9	1.8	9.47*	.35*	17.0*	Blight
3	Val/RL	10	0	0.30	1.05	24.0	—
		8	1.6	20.16*	.18*	20.4	Blight
4	Tom/RL	3	0	1.75	1.73	—	—
		3	1.8	16.50	.22*	—	Blight
5	Tom/RL	4	0	0.88	.81	—	—
		4	1.5	9.94	.19*	—	Blight
6	Val/ ?	3	0	0.83	2.34	—	—
		3	2.2	17.00*	0.00*	—	Blight
7	Val/RL	10	0	1.05	2.43	157.5	—
		10	2.2	7.95*	.07*	21.0*	Blight
Eastern Transvaal							
8	Gft/RL	10	0	0.75	.38	—	—
		10	1.9	9.83*	0.00*	—	Blight
9	Val/RL	10	0	1.03	.81	—	—
		10	1.8	13.50*	0.00*	—	Blight
10	Val/RL	10	0	1.35	2.00	—	—
		10	1.9	15.20*	0.00*	—	Blight
11	Val/RL	9	0	3.22	1.35	—	—
		11	2.3	13.77*	0.00*	—	Blight

TABLE 1 (CONT'D)
SUMMARY OF VISUAL RATINGS, ZINC ACCUMULATION IN TRUNK WOOD, AND WATER UPTAKE IN DECLINED AND HEALTHY TREES TESTED FOR THE PRESENCE OF CITRUS BLIGHT IN DIFFERENT AREAS OF SOUTH AFRICA

Grove no.	Scion/stock†	No. of trees	Visual rating‡	Zinc (μg/g)	Water uptake syringe (ml/sec)	Water uptake bottle (ml/day)	Diagnosis§
12	Val/RL	5	0	1.85	1.05	111.0	—
		3	2.8	9.25*	0.0	8.3	Blight
13	Val/RL	20	0	1.25	1.51	183.3	Greening
Natal							
14	Val/RL	10	0	1.63	1.54	—	—
		10	1.7	8.84*	.39*	—	Blight
15	Val/RL	4	0	1.06	1.06	—	—
		4	2.3	1.94	1.21	—	Unknown
16	Val/RL	5	0	2.70	2.63	—	—
		5	1.6	5.10	1.51	—	Unknown
Eastern Cape Province (Sundays River Valley)							
17	EL/RL	10	0	1.30	1.44	—	—
		10	3	2.23	1.57	—	Unknown
18	EL/RL	5	0	1.35	1.40	—	—
		5	3	1.75	2.44	—	Unknown
19	Val/RL	8	0	1.78	.55	—	—
		8	3	3.66*	.07*	—	Blight
20	Navel/RL	2	0	1.25	.45	—	—
		2	3	5.50	0.00*	—	Blight

TABLE 1 (CONT'D)
SUMMARY OF VISUAL RATINGS, ZINC ACCUMULATION IN TRUNK WOOD, AND WATER UPTAKE IN DECLINED AND HEALTHY TREES TESTED FOR THE PRESENCE OF CITRUS BLIGHT IN DIFFERENT AREAS OF SOUTH AFRICA

Grove no.	Scion/stock†	No. of trees	Visual rating‡	Zinc (μg/g)	Water uptake syringe ml/sec)	Water uptake bottle (ml/day)	Diagnosis§
Swaziland							
21	Val/RL	10	0	0.50	1.24	109.4	—
		10	2.0	13.65*	.10*	17.0*	Blight
		10	3.0	16.40*	0.00*	—	
22	Val/RL	3	0	0.92	.96	—	—
		3	2.2	13.83*	0.00*		Blight

† Val. = Valencia, RL = rough lemon, Tom = tomango, Gft = redblush grapefruit, and EL = Eureka lemon.
‡ Tree canopy condition was rated on a scale whereby 0 = healthy and 3 = severe decline. The apparently healthy trees were grouped together and compared to the declined trees when running the diagnostic tests for citrus blight.
§ The cause of decline was diagnosed as citrus blight when the declined trees had characteristic foliar symptoms of blight, accumulation of zinc in trunk wood, reduced water uptake, and the presence of amorphous type plugs. If these conditions were not met, the decline was listed as unknown decline, except in grove number 13 where trees were affected with citrus greening.
* Values are significantly different from the healthy trees at that location as determined by Fisher's least significant difference test, P = 0.05.

toms characteristic of blight, the tests for blight did not confirm this. In the SRV of Eastern Cape Province, declining Eureka lemon trees (Groves 17 and 18) did not have zinc accumulation in the trunk wood or reduced water uptake. The Valencia and Navel trees in this area (Groves 19 and 20) did have high zinc in trunk wood and a significant reduction of water uptake to indicate that blight is present.

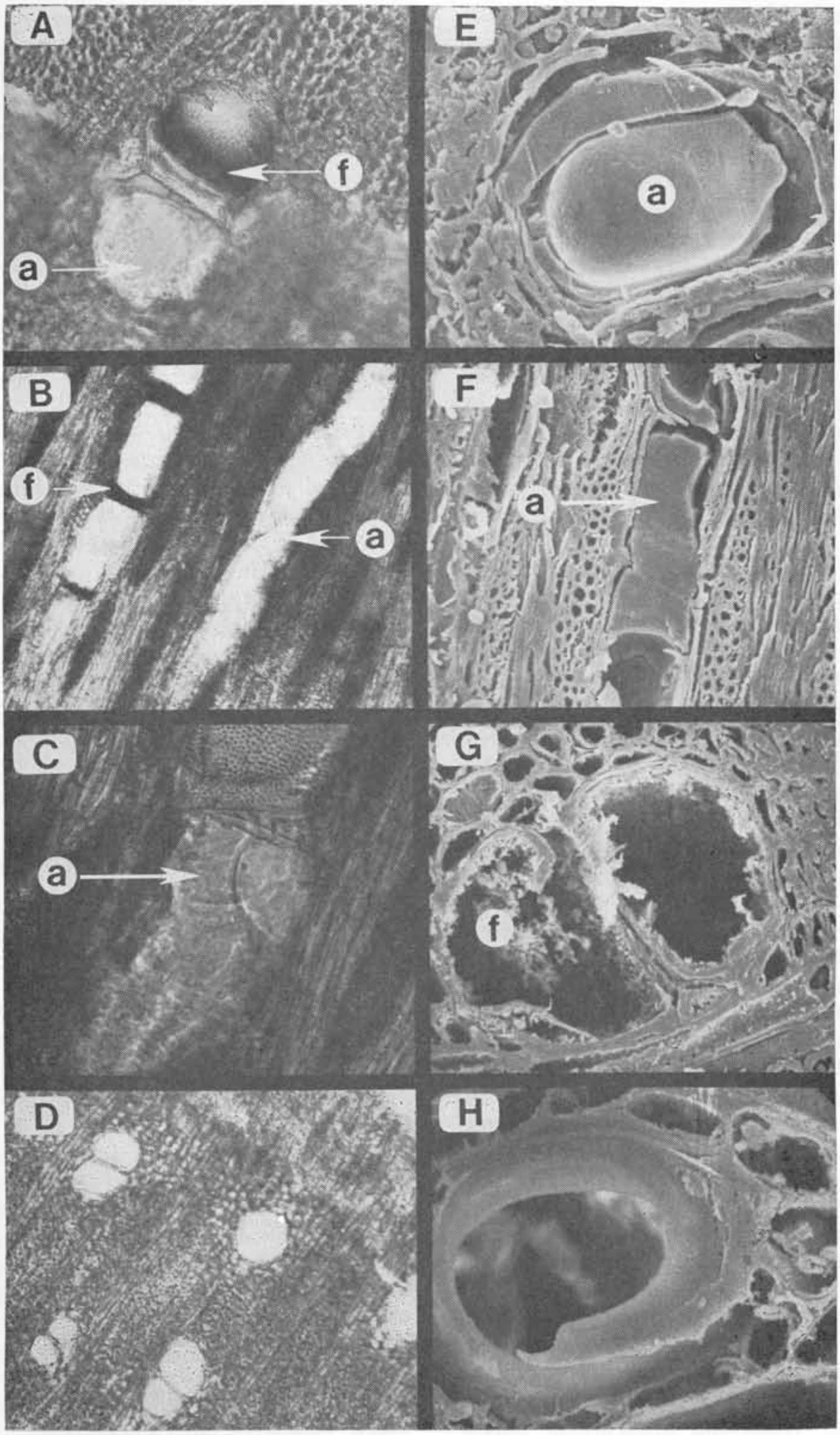
Blight is more prevalent in the warmer areas of South Africa. In the Northern Transvaal and Eastern Transvaal, many groves had blight-like symptoms which made it easy to select groves to test. In the cooler areas, however, it was difficult to locate groves having blight-like symptoms and, even then, only one of the three groves tested in Natal and two of the four groves tested in Eastern Cape Province had blight. In groves having blight, trees with sectored symptoms (on only one side of the canopy) had the severest and earliest symptoms on the northwest sector; the side most exposed to the sun and the warmest side of the tree.

Cytological examination of core samples from Northern Transvaal, Eastern Transvaal, and Swaziland where the decline trees had low water uptake and high zinc levels in the trunk xylem revealed that amorphous plugs were common in the xylem vessels of decline trees (fig. 1). Few plugs were present in the xylem of healthy trees and these were usually the fibrous plugs, which were also present in decline trees (fig. 1).

Core samples from the declining Eureka lemon on rough lemon in the SRV (Grove 18) contained numerous amorphous plugs which lined the xylem vessels, but these differ from the amorphous plugs found in blight trees in that they did not plug the vessels completely (fig. 1H). This type of plug was not present in the healthy trees in the same grove. These trees were not affected by blight as there was no significant difference in water uptake or zinc levels in trunk xylem between healthy and decline trees.

It has recently been reported in Florida that trees severely affected with blight often have a high percentage of the major roots with root necrosis while trees mildly affected by blight, healthy trees, and trees recently defoliated by freezing have very few lesioned roots (8). We examined the root systems of healthy and declining trees at several locations to see if root necrosis was associated with the decline in South Africa. In all the areas except the SRV in Eastern Cape Province, less than 10% of the major roots were lesioned. However, all the trees examined in the SRV (Groves 17 through 20) had severe necrosis on the major roots with greater than 90% of the root system being affected on both severe decline and mild decline trees. On the trees examined in the SRV, the feeder root system was greatly reduced due to complete necrosis of most major roots. The wood beneath the cambial layer was stained a dark purple color.

Earlier work by da Graca and van Vuuren (7) indicated a lack of correlation between stem pitting on rough lemon rootstock and blight-like decline in the Transvaal. We examined the trees in several groves tested for blight for the occurrence and severity of stem pitting above and below the budunion. Stem pitting was often found on the rough lemon rootstock on both apparently healthy trees and declining trees. This stem pitting appeared as small, teeth-like projections from the cambial face of the bark which fit into corresponding depressions in the xylem. Stem


A
f
a
E
a
B
f
a
F
a
C
a
G
f
D
H

pitting on the rough lemon rootstock occurred in five of seven groves (Groves 1, 4, 5, 6, and 7) in Northern Transvaal, four of four groves 8, 9, 10, and 11) in Eastern Transvaal, three of three groves in Natal, two of four groves (Groves 19 and 20) in Eastern Cape Province, and one of two groves (Grove 22) in Swaziland. In Groves 1 and 4 in the Northern Transvaal, the most severe stem pitting was found. The rest of the groves having stem pitting were relatively mildly affected with the rating ranging from 0.1 to 2.0, averaging 0.6.

DISCUSSION

Citrus blight occurs in South Africa and Swaziland as determined by the tests presently available to diagnose blight. There is a greater incidence of blight in the warmer growing areas of citrus. While blight was not as prevalent in the cooler areas of Natal and Eastern Cape Province, many trees were declining due to greening disease which is prevalent in these areas (12). Trees affected only with citrus greening had zinc levels and water uptake similar to healthy trees. Tests for citrus blight therefore were not affected by the presence of citrus greening disease. Previous reports also have shown that citrus trees affected by greening, tristeza, and other citrus viruses, insect, fungal, nematode, and physiological problems all have trunk wood zinc levels similar to that of healthy trees (1, 7, 13).

The low incidence of root necrosis on the major roots of healthy and decline trees in all areas except SRV indicates that root necrosis is not involved in the blight syndrome. This supports the conclusion of Graham **et al.** (8) in Florida that root necrosis is not prevalent in mildly affected blight trees but develops only after the tree has been severely affected. In the SRV, root necrosis resembling dry root rot of citrus as described by Bender (2) is prevalent in groves without blight (Groves 17 and 18) as well as groves with blight (Groves 19 and 20).

Stem pitting on rough lemon rootstocks is common in many of the citrus growing areas of South Africa and Swaziland. The cause of stem pitting or its effect on citrus was not determined. However, further studies on stem pitting are underway to determine the role of citrus tristeza virus or other citrus viruses.

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Fig. 1. Light (A-D) and scanning electron microscopy (E-H) of plugging materials in xylem vessels from declining and healthy citrus trees. A. Transverse section of xylem vessels from a blight-affected tree showing one vessel completely plugged with an amorphous plug (a) and another vessel partially occluded with a fibrous plug (f). Note dark color of fibrous plug and fibrous nature in lumen of vessel (X207). B. Longitudinal section of vessels from a blight-affected tree with dark colored fibrous plugs (f) at the junctions of vessels. Amorphous plugs (a), which naturally appear yellow, completely occlude the vessel on the right (X84). C. Higher magnification of an amorphous plug (a) in a longitudinal section from a blight-affected tree (X210). D. Transverse section of the xylem from a healthy citrus tree which has good water conductivity and lacks plugs (X84). E. Scanning electron micrograph of an amorphous plug (a) in transverse section from a blight-affected tree. Note the plug totally occludes the vessel (X420). F. Scanning electron micrograph of an amorphous plug (a) in a longitudinal section from a blight-affected tree (X154). G. Transverse section of vessels from a blight-affected tree containing fibrous plugs (f). Note how walls are lined with the fibrous plug. Scanning electron micrograph (X420). H. Scanning electron microscopy of a xylem vessel in transverse section from a Eureka lemon affected with an unknown decline in the Sunday's River Valley. Amorphous type plugs occurred but did not completely occlude the vessels (X658).

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