Pathogenicity of Two Biotypes of Elsinoë fawcetti to Sweet Orange and Some Other Cultivars

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ABSTRACT

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All 24 single-conidium isolates of *Elsinoë fawcetti* obtained from susceptible cultivars of citrus in different Florida groves and nurseries were pathogenic to rough lemon shoots, grapefruit shoots and fruit, and Murcott fruit; but only 13 of them incited scab on sour orange shoots. It has been customary to refer to scab caused by *E. fawcetti* as sour orange scab but this needs revision in the light of these findings. None of the isolates was pathogenic to shoots of sweet orange. Fruits of sweet orange and Temple were infected only by the 13 isolates that were pathogenic to sour orange shoots. In the field, scab is very common and often severe on fruit of the Temple cultivar but it seldom appears on sweet orange, even though both cultivars are susceptible to the same biotype of *E. fawcetti*. The rare occurrences of scab on sweet orange fruit apparently is due, at least partly, to the poor chances for inoculum carry-over in the tree canopy from one crop to the next, rather than to any high inherent resistance of the fruit rind to infection.

Additional key words: *Sphaceloma fawcetti*, production of inoculum, inoculation technique.

In Florida, only one citrus scab-inducing fungus, *Elsinoë fawcetti* Bitanc. and Jenkins, has been recorded (2, 4) and it is known only in its imperfect state, *Sphaceloma fawcetti* Jenkins.

Following the discovery in Brazil in 1937 (1) of another citrus scab-inducing fungus, *Elsinoë australis* Bitanc. and Jenkins, it has been customary to call the disease caused by *E. fawcetti*, sour orange (*Citrus aurantium* L.) scab and that caused by *E. australis*, sweet orange (*C. sinensis* Osbeck) scab. This was considered appropriate because *E. australis* caused severe attacks on sweet orange and failed to infect sour orange (1). Conversely, sour orange scab was considered an appropriate name for the scab disease caused by *E. fawcetti* because of the severity and common occurrence of the disease on sour orange fruit and foliage (1, 2).

In Florida, scab is observed only very occasionally on sweet orange rind (2, 4, 5). In 1923, Winston et al. (8) reported some rare occurrences of the disease on Lue Gim Gong (a Valencia sweet orange type), Maltese, Mediterranean sweet, Pineapple, and Washington navel sweet orange cultivars. Winston (7) failed to obtain infection of sweet orange fruit by artificial inoculation with any isolates of the fungus obtained from sweet orange and other susceptible cultivars but he was successful in obtaining infection of grapefruit with such isolates.

To this day, scab has continued to appear only very occasionally on sweet orange fruit in Florida, and the attacks usually have been too mild to cause concern. However, some relatively severe outbreaks of scab occurred on sweet orange in Collier and St. Lucie Counties in 1967 and 1968. In one 4-yr-old planting of Hamlin sweet orange on rough lemon (*C. jambhiri* Lush.) rootstock in Collier County in 1967, 4% of the fruit showed scab, but the disease has not reappeared in this grove since 1968. In St. Lucie County, in 1968, 20% of fruit showed scab in one planting of Washington navels, and 5% of fruit showed symptoms in nearby plantings of Hamlin and Valencia orange, but again the disease has not reoccurred since that year.

In 1975, there was another noteworthy record of scab on sweet orange fruit; this time on container-grown Valencia trees in a Polk County nursery.

This paper describes procedures for inoculating fruit and shoots with conidial inoculum derived from single-conidium isolates of *E. fawcetti* cultured on artificial media. Results are given for inoculations of sweet orange and some other citrus cultivars using isolates obtained from different hosts and locations in the Florida citrus belt. An important aim of the studies was to try to explain why scab, which is common in Florida on several susceptible cultivars, only rarely appears on sweet orange.

MATERIALS AND METHODS

Fungal isolates.—The scab pathogen was isolated from sour orange, Temple (*C. temple* Hort. ex Y. Tanaka),

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Valencia and Hamlin sweet orange, Marsh grapefruit (*C. paradisi* Macf.), Murcott (? *C. reticulata* Blanco hybrid), rough lemon, Milam (? rough lemon hybrid), lemon [*C. limon* (L.) Burf. f.], Carrizo citrange [*C. sinensis* × *Poncirus trifoliata* (L.) Raf.], Orlando tangelo (*C. paradisi* × *C. reticulata*) and Page (Minneola tangelo × *C. reticulata*) (Table 1). The two sweet orange isolates from St. Lucie County were obtained by M. Cohen, Agricultural Research Center, Fort Pierce, Florida, from groves infected in 1968. All other isolates were obtained from diseased material collected between 1972 and 1976. One of the isolates originated from the Valencia fruit infected in the Polk County nursery in 1975.

Isolation procedure.—Isolation of the causal fungus from lesions was difficult because of its very slow growth and confinement to the outer scabby portion of the eruptions that is usually heavily contaminated by other organisms. Scab pustules were swabbed with 95% alcohol to remove at least some of the contaminating organisms. Thin tangential sections then were cut with a scalpel from the scabby tissue and deposited in a sterile petri dish. Sections were mashed with a bent steel spatula and the resulting fragments were dispersed in water agar that was poured into the dish. This procedure was intended to produce pieces that were so minute that at least some of them might contain the pathogen alone. After 3-4 days at 25 C, the margins of the fragments were viewed with ×100 magnification for presence of the characteristic hyphal growth of *E. fawcetti*. Portions of such growth were transferred for culture and storage on potato-dextrose agar (PDA) prior to the production of single-spore cultures.

An unusually elaborate procedure was necessary to obtain single-conidium isolates of *E. fawcetti*. This was necessitated by the small size of the conidia, by the fact that the conidia are produced only when mycelium is starved and submerged in water, by the very gelatinous nature of the colonies and conidia surface and by the tendency for conidia to sink rapidly after liberation and adhere to any glass surface. A portion of the colony in PDA was transferred to a 100-mm diameter petri dish and mashed into small fragments. The fragments were dispersed in 10 ml of Fries' solution (6), that was poured into the dish. Then a microscope slide was placed in the dish. Suspended fragments became affixed to the slide and produced slow-growing colonies (microcolonies). After 3-4 days at 25 C, the slide, with microcolonies attached, was rinsed three times in sterile water to remove remaining nutrients and transferred to another petri dish. Distilled water was added until the slide was barely submerged (deeper immersion would have inhibited production of conidia). After production of conidia had commenced, the petri dish was placed on a mechanical shaker to prevent immediate settling of conidia and to disperse at least some of them beyond the edge of the slide. Then conidia were allowed to settle and adhere to

TABLE 1. Pathogenicity of 24 Florida isolates of *Elsinoë fawcetti* to various citrus species and cultivars

	Source of isolate		Resistance (R)/susceptibility (S) of part of citrus cultivar inoculated							
			Sweet orange		Sour orange	Temple	Rough lemon	Grapefruit		Murcott
Cultivar	Part	Location[a]	fruit[b]	shoots[c]	shoots	fruit	shoots	fruit	shoots	fruit
Sour orange	rootsprout	Collier (G)	S*	R	S	S	S	S	S	ND
Temple	fruit	Polk (G)	S*	R	S	S	S	S	S	S
Temple	leaf	Polk (G)	S*	R	S	S	S	S	S	S
Temple	fruit	Polk (N)	S	ND[d]	S	S	S	S	ND	S
Temple	fruit	Lee (N)	S	R	S	S	S	S	S	S
Valencia orange	fruit	Polk (N)	S*	R	S	S	S	S	S	S
Valencia orange	fruit	St. Lucie (G)	S*	R	S	S	S	S	S	ND
Hamlin orange	fruit	St. Lucie (G)	S*	R	S	S	S	S	S	S
Marsh grapefruit	fruit	St. Lucie (G)	S	R	S	S	S	S	S	S
Marsh grapefruit	fruit	Lee (N)	S	R	S	S	S	S	S	S
Marsh grapefruit	fruit	Polk (G)	R*	R	R	R	S	S	S	S
Marsh grapefruit	fruit	Polk (G)	R*	R	R	R	S	S	S	ND
Marsh grapefruit	fruit	Polk (G)	R	R	R	R	S	S	S	S
Marsh grapefruit	fruit	Polk (G)	R	ND	R	R	S	S	ND	S
Marsh grapefruit	fruit	St. Lucie (G)	R	R	R	R	S	S	S	S
Murcott	fruit	Polk (N)	S	R	S	S	S	S	S	S
Murcott	fruit	Polk (G)	R	ND	R	R	S	S	ND	S
Rough lemon	leaf	Polk (N)	R*	R	R	R	S	S	S	S
Milam	leaf	Polk (N)	R	ND	R	R	S	S	ND	S
Lemon	fruit	Martin (G)	R*	R	R	R	S	S	S	S
Carrizo citrange	leaf	Polk (N)	S	ND	S	S	S	S	S	ND
Carrizo citrange	leaf	Highlands (N)	S	R	S	S	S	S	S	S
Orlando tangelo	fruit	Polk (G)	R	R	R	R	S	S	S	S
Page	fruit	Hillsborough (G)	R	ND	R	R	S	S	ND	S

[a]Name of county and whether from nursery (N) or grove (G).
[b]All isolates were tested on Valencia. Those marked with asterisk (*) were also tested on Pineapple sweet orange with the same results.
[c]All isolates were tested on shoots of Pineapple sweet orange.
[d]ND = no data obtained because of loss of conidia production by isolate before these inoculations were made.

the bottom of the dish. The water was decanted and 10 ml of PDA was poured into the petri dish. After 2 days a microcolony that was observed to have originated from a single conidium was transferred to a PDA slant to provide the stock culture for the pathogenicity tests.

Preparation of inoculum.—Initially, the pathogenicity of different isolates of *E. fawcetti* was tested by using fragmented colonies derived from PDA cultures as described by Winston (7) and Bitancourt and Jenkins (1), but the results were inconsistent and often negative, even on the highly susceptible Temple cultivar. Fragmented mycelia from PDA cultures sometimes produced conidia, but only in small numbers. Later, I found that infection of susceptible host tissue could be assured only when the inoculum contained abundant conidia.

The following procedure was used to produce conidia for inoculum. A portion of a 3-to 4-wk-old colony growing on PDA was transferred to a 100-mm diameter petri dish, mashed, and dispersed in 10 ml of Fries' solution. After 3 days at 25 C, the medium was decanted, leaving the numerous, but still nonsporulating, microcolonies still attached to the bottom of the dish. The dish was flushed three times with water and water then was added until the microcolonies were barely covered. After 8-10 hr at 25 C, the microcolonies, as well as conidia produced by this time and attached to the bottom of the dish, were dislodged with a small camel's-hair brush and suspended in 50 ml of distilled water.

Production of conidia continued from microcolonies and mycelial fragments following their deposition on inoculated leaves and fruit. Numbers of conidia also were increased by budding. Because the ultimate number of infection propagules produced would not be known, no attempt was made to standardize the inoculum suspension.

Inoculation.—Pathogenicity of isolates to shoots (young leaves and stems) was determined in the greenhouse on container-grown plants that had been pruned to promote new susceptible growth. After shoots appeared, but before all their leaves were visible, the plants were held upside down and the new growth was immersed in the inoculum. The plants were enclosed immediately with polyethylene bags to prevent evaporation of the liquid water which is essential for conidia germination and infection (6). The bags were removed after 2 days and first symptoms appeared 2 days later. The inoculum was applied to only one plant of each cultivar at each time of testing and at least two tests were made with each isolate.

Fruit inoculations were conducted outdoors on trees that previously had shown little or no scab. Most fruit were inoculated 4-8 wk after petal fall. This was a compromise based (i) on previous findings that fruit of the highly susceptible Temple cultivar become immune to attack 10-12 wk after petal fall (Whiteside, *unpublished*) and (ii) on the fact that after the first 4 wk, the normal fruit-drop-thinning process tends to slow down, thus improving the chances that inoculated fruit would remain on the tree. Inoculum was applied to 8-10 fruit of each cultivar in each test. On the day of inoculation a 30×5 cm strip of absorbent cotton was wrapped around 6-8 cm of the fruit stalk next to the calyx and around the whole fruit except for a small area at the stylar end which was left exposed. Subtending leaves, when sufficiently close to the fruit, also were enclosed with the cotton. This helped to preserve a film of water on the fruit surface. Scab tended to be more severe where a leaf had been in contact with the fruit surface during the inoculation period than where the rind had been in contact only with cotton.

Prior to inoculation, the wrapped fruit was immersed in water to saturate the cotton thoroughly. After dripping had ceased, the fruit was held with its stylar end uppermost and 10 ml of inoculum were poured onto the exposed rind. Fruit inoculations were delayed until 1-2 hr before sunset to improve the chances for infection. The cotton wraps were removed the next afternoon and symptoms appeared within 1 wk.

Pathogenicity tests were made on shoots of rough lemon, sour orange, Pineapple sweet orange, and Marsh grapefruit, and on fruit of Valencia and Pineapple orange, Marsh grapefruit, Temple, and Murcott. All isolates were tested at least twice on each cultivar and inoculum virulence was checked by including a known suscept, usually rough lemon, in each test.

RESULTS AND DISCUSSION

Morphologically, all the isolates listed in Table 1 fitted the imperfect state of *E. fawcetti*. This was based on the fact that all scab lesions derived from them on inoculated rough lemon shoots produced the two asexual spore forms described by Jenkins (3) as characteristic of *Sphaceloma fawcetti*; hyaline elongate conidia and colored spindle-shaped conidia. Production of the hyaline conidia was promoted merely by wetting the scab lesions for a minimum of 2-4 hr. The colored conidia were produced only after exposing the plants with scab pustules outdoors for 1-2 wk.

Fig. 1. Symptoms of scab on Valencia orange following inoculation with the sour orange-, Temple-, and sweet orange-infecting biotype of *Elsinoë fawcetti*.

It had been speculated that the occasional attacks of scab on sweet orange in Florida might have been caused by *E. australis*, the sweet orange scab fungus. Knorr (4) considered the scab eruptions on sweet orange in Florida to differ from those of sweet orange scab in South America and he therefore discounted *E. australis* as a possible cause. His contention was supported by the fact that *E. australis* produces only hyaline conidia (1) and not the colored conidia characteristic of *E. fawcetti*, which all isolates used in this study produced.

A few isolates lost their capacity to produce conidia before all the pathogenicity tests were completed (Table 1). Despite the omissions in the data thus incurred, there was clear evidence for the existence of at least two biotypes of *E. fawcetti* in Florida. One biotype was pathogenic to Marsh grapefruit shoots and fruit, Murcott fruit, and rough lemon shoots but not to sour orange shoots and sweet orange and Temple fruit. The other biotype was pathogenic to all these cultivars. Neither biotype was pathogenic to sweet orange shoots. Marsh grapefruit shoots showed a relatively mild reaction to inoculation compared with the shoots of sour orange and rough lemon, both with respect to numbers of pustules produced and their size.

Spectacular scab eruptions were produced on Valencia orange fruit following artificial inoculation with isolates that infected sour orange shoots and Temple fruit (Fig. 1). These results differ from those obtained by Winston (7), who was unable to incite scab on sweet orange fruit even with isolates from sweet orange. Winston used inoculum derived from colonies on PDA. Such colonies would have had relatively little conidia-producing capability and hence little or no infective ability.

The possibility that my inoculation technique was too severe to appraise the field susceptibility of sweet orange to naturally produced inoculum is discounted by the following observations: (i) pronounced scab eruptions have appeared on sweet orange rind in some of the groves where natural infection occurred, and (ii) severe symptoms of scab appeared on sweet orange fruit after freshly-collected, scab-infected Temple shoots were placed above them in the tree canopy and overhead sprinkler irrigation was applied for 6 hr to disperse the conidia and provide an infection period.

The sweet orange-infecting biotype of *E. fawcetti* apparently is widespread in Florida citrus groves and nurseries based on the common occurrence of scab on sour orange and the Templer cultivar. The fact remains, however, that scab rarely appears on sweet orange. A major reason for this may be the absence of shoot infection on sweet orange trees.

Without shoot infection, the fungus can survive on the tree only on diseased fruit. However, there is some doubt as to how long scab pustules on fruit rind can retain a capacity for production of conidia. Winston (7) concluded from field observations and fungal isolations that the pathogen seldom, if ever, overwinters on fruit formed the previous spring. In contrast, he observed that the pathogen could survive in infected leaves for as long as 12 mo. Thus, even if no infection occurred later in the year on summer or early-fall flushes of growth, it would still be possible for the fungus to survive on shoots in the tree canopy from one year to the next. Feasibly, infected fruit derived from late out-of-season bloom might pose a threat to the following fruit crop if they remained on the tree long enough. Such out-of-season cropping can be heavy on Temples and lemons, but is comparatively rare on sweet orange.

There remains doubt as to the sources of inoculum that caused the 1967-1968 scab outbreaks on sweet orange in St. Lucie and Collier Counties. In all cases the trees were growing on scab-susceptible rootstocks (rough lemon and sour orange). However, no rootsprouts were seen on the diseased trees that might have provided the inoculum: at least, not when the disease was first noticed, which was 4-6 mo after the infection would have occurred. The source of inoculum for the attack on the container-grown Valencia orange fruit in a Polk County nursery in 1975 appeared more certain, because the trees were situated among heavily infected Temple trees.

Wind dispersal of colored conidia of *E. fawcetti* has been demonstrated (6). Thus, it would be feasible for conidia to reach sweet orange trees from nearby infected trees. Generally, however, scab epidemics seem to result from the production of abundant water-dispersed, hyaline conidia that have their greatest impact within the canopy of origin. The opportunities for massive movement of inoculum from one grove to another are poor. Dispersal of a few conidia to another grove is likely to lead to an epidemic only if conditions are exceptionally favorable for abundant secondary infections during the short time that the fruit remains susceptible. Generally, conditions in Florida during the critical scab-susceptible period are too dry for this to occur.

The results of the inoculation tests indicate that the scarcity of scab on sweet orange in Florida is due to an inability of the fungus to survive in the tree canopy and to reach the trees from infected neighboring groves and not to any inherent high resistance of the rind to infection by *E. fawcetti*.

An unexpected outcome of these studies was that sour orange was susceptible to only 13 of the 24 isolates tested. Therefore, it is misleading to refer to the disease caused by *E. fawcetti* as sour orange scab. A return to the name, citrus scab, as used in most of the earlier Florida reports on this disease (5, 7, 8) is deemed necessary.

LITERATURE CITED

1. BITANCOURT, A. A., and A. E. JENKINS. 1937. Sweet orange fruit scab caused by Elsinoë australis. J. Agric. Res. 54:1-18.
2. FAWCETT, H. S. 1936. Citrus diseases and their control. McGraw-Hill, New York and London. 656 p.
3. JENKINS, A. E. 1931. Development of the citrus-scab organism, Sphaceloma fawcetti. J. Agric. Res. 42:545-558.
4. KNORR, L. C. 1973. Citrus diseases and disorders. The University Presses of Florida, Gainesville. 163 p.
5. RUEHLE, G. D., and W. L. THOMPSON. 1939. Commercial control of citrus scab in Florida. Fla. Agric. Exp. Stn. Bull. 337. 47 p.
6. WHITESIDE, J. O. 1975. Biological characteristics of Elsinoë fawcetti pertaining to the epidemiology of sour orange scab. Phytopathology 65:1170-1175.
7. WINSTON, J. R. 1923. Citrus scab: its cause and control. U.S. Dep. Agric. Bull. 1118. 38 p.
8. WINSTON, J. R., J. J. BOWMAN, and W. J. BACH. 1925. Relative susceptibility of some rutaceous plants to attack by the citrus-scab fungus. J. Agric. Res. 30:1087-1093.