Factors Contributing to the Rare Occurrence of Scab on Sweet Orange in Florida

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ABSTRACT

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Despite the common occurrence in Florida of a virulent sweet orange-infecting biotype of *Elsinoë fawcettii*, scab rarely appears in solid plantings of sweet orange. Because sweet orange foliage is immune to scab, the pathogen can survive in sweet orange groves only if there are diseased rootsprouts present from scab-susceptible rootstocks, or if inoculum-bearing fruit remain on the tree until after the next bloom. Viable propagules of *E. fawcettii* were detected in the stromatic portion of scab pustules on inoculated Valencia orange fruit for the first 6–9 mo after infection, but few or none were detected after 10–12 mo. No scab appeared on fruit that set from the spring bloom near 12- to 14-mo-old diseased Valencia fruit. The disappearance of scab from previously infected sweet orange groves is attributed to a loss of inoculum-producing ability of the pustules on the previous year's in-season fruit before the next crop becomes exposed to infection and to a paucity of out-of-season late-set fruit which, if they became infected, might provide inoculum to infect the next in-season crop of fruit.

In Florida, scab caused by *Elsinoë fawcettii* Bitanc. & Jenk. is common on several citrus cultivars including rough lemon (*Citrus jambhiri* Lush.), sour orange (*C. aurantium* L.), grapefruit (*C. paradisi* Macf.), and Temple (*C. sinensis* (L.) Osbeck × *C. reticulata* Blanco), but it is rare on sweet orange (*C. sinensis*) (2,5,7). Sweet orange fruit have been considered highly resistant or even immune to *E. fawcettii* (1,2,7), but when the rind is invaded by this pathogen, either by natural infection or artificial inoculation, typical scab symptoms are produced (5).

One of the two biotypes of *E. fawcettii* found in Florida is pathogenic to sweet orange, and only this one is pathogenic to Temple and sour orange (5). Scab is common and widely distributed in Florida on Temples in groves and nurseries, and on sour orange rootstock seedlings in nurseries. Therefore, the rare occurrence of scab on sweet orange cannot be attributed to the local absence of a virulent scab-causing pathogen.

Both the leaves and fruit of Temple trees are susceptible to *E. fawcettii*, whereas only the fruit of sweet orange trees are infected (5). Thus, in sweet orange groves, the only means of pathogen carry-over would be on infected fruit, on infected rootsprouts from a scab-susceptible rootstock, or in the canopy of nearby trees of a susceptible cultivar.

The chances for fruit-to-fruit infection on early and midseason cultivars of sweet orange are remote because the mature crop is usually picked before the next year's bloom, and because out-of-season fruit development is uncommon in Florida on sweet orange trees. Only on the late-maturing Valencia cultivar do two crops commonly overlap.

In previous field studies on the pathogenicity of different isolates of *E. fawcettii* to sweet orange fruit (5), some infection was observed of newly set fruit on one of 12 trees that carried artificially infected fruit from the previous year. However, there was no recurrence of the disease in later years,

which suggested that the pathogen had a limited ability to survive through the following winter and spring and to infect the next crop of fruit. The objective of this study was to determine how long *E. fawcettii* could survive in scab pustules on sweet orange fruit.

MATERIALS AND METHODS

Because natural outbreaks of scab on sweet orange are rare in Florida, a supply of diseased fruit could be assured only through artificial inoculation. The method used for inoculum production was essentially the same as the one previously described (5). Isolates of *E. fawcettii* were obtained from scab pustules on Temple fruit. A portion of an actively growing colony of this fungus on potato-dextrose agar (PDA) was transferred to a sterile 90-mm-diameter petri dish and mashed with a bent steel spatula. The resulting fragments were dispersed in 10 ml of Fries' solution that was poured into the dish. Fungal colonies derived from these fragments adhered firmly to the bottom of the dish. After 3 days at 25 C the medium was decanted, leaving the colonies attached to the dish. The dish was flushed three times with distilled water. Finally, enough water was poured into the dish to barely cover the fungal growth. Conidia, which began to form after about 6 hr, separated from the colonies, sank, and also became attached to the bottom of the dish. Conidia were seldom produced in distilled water and erratic results were encountered with deionized water. This was partly overcome by using a 0.02-M potassium phosphate buffer (pH 7.1). In the last year of the study, autoclaved water from a local lake (pH 7.2–7.4) was used and this consistently promoted heavier conidial production than the phosphate buffer. After keeping the dishes at 25 C for 18–24 hr, the fungal colonies and conidia were dislodged with a camel's-hair brush and suspended in distilled water (50 ml per 90-mm-diameter dish).

Young Valencia orange fruit were inoculated individually at 4–8 wk after petal fall using a technique similar to that previously described (5). Each year, 50–60 fruit were inoculated per week over a 4-wk period in May. The fruit that were inoculated were located on the lower part of the canopies of 12 trees. More fruit were inoculated than needed, to compensate for losses from natural abscission, which continued to occur until fruit were more developed. A 30 × 5 cm strip of absorbent cotton was wrapped around each fruit and part of its stem. All subtending leaves were retained, and those enclosed by the wrap were orientated with their blades flat against the fruit surface. The stylar end of each fruit was left exposed. The cotton was saturated with water and the part below the fruit was gently squeezed to hold it around the stem. Each fruit was held with its stylar end up, and about 10 ml of inoculum was poured over the exposed rind. Most infection occurred where the leaf blades were in contact with the rind surface, probably because of longer retention of water at these locations. Inoculations were made shortly before sundown to avoid rapid drying, and the wraps were removed before noon the next day to avoid overheating.

At various intervals after inoculation fruit were picked and tested for their ability to produce inoculum. This could not be achieved by wetting the pustules to promote production of conidia, because the conidia could not be distinguished with certainty from those of contaminants. Therefore, the pathogen had to be isolated from the scab stromata and cultured on PDA to produce a characteristic and easily identified fungal colony. The assumption was made that if any viable mycelia or conidiophores remained on or in the scab pustule there could still be a potential for further production of conidia if the fruit were wetted by rain, dew, or overhead sprinkler irrigation.

Elsinoë fawcettii grows so slowly on agar media that it is often quickly overgrown by other organisms present in the stromata, particularly when isolations are attempted from older pustules. The following medium, although only semiselective, helped to solve this problem. It consisted of 39 g of PDA, 100 mg of streptomycin sulfate, 100 mg of tetracycline hydrochloride, and 400 mg a.i. of dodine (Cyprex 65 W) per liter of water, and was prepared as previously described (6).

A diseased fruit was held above an open petri dish containing the medium and minute fragments were scraped from the scab pustules and allowed to fall and scatter as uniformly as possible over the agar surface. From each fruit, the fragments were scraped from a combined area of about 2 cm^2 of scabby surface and dispersed over four 90-mm-diameter petri dishes. Fruit were not surface-sterilized because this would have killed any propagules of *E. fawcettii* on the surface of the stromata. In the third test (1986–1987), an attempt was made to quantify the residual viability of *E. fawcettii* in scab pustules by counting the number of colonies formed on each petri dish. However, this provided only an approximate estimate of the residual potential activity of the pustules because the number and size of the fragments settling on the medium were not accurately controlled. Uniformity of deposition was judged visually. The number of fruit sampled at each time of testing is given in Table 1.

To monitor the accuracy of the isolation technique used at each time of sampling, a parallel series of scrapings was made from scab pustules on 4- to 10-mo-old Temple leaves onto the semiselective, dodine-amended PDA.

A search was made for any natural spread of the fungus from diseased fruit

Table 1. Detection of viable propagules of *Elsinoë fawcettii* in the stromata of scab pustules on Valencia orange fruit at various times after infection

Period from inoculation to sampling (mo)	May 1982		May 1984[a]		May 1986		
	Fruit sampled (no.)	Fruit yielding propagules (%)	Fruit sampled (no.)	Fruit yielding propagules (%)	Fruit sampled (no.)	Fruit yielding propagules (%)	Colony-forming units per dish (no.)[b]
5	...[c]	...	10	100	4	100	ND[d]
6	...	...	20	50	12	100	37.1
7	12	100	20	60	4	100	41.2
8	12	100	20	40	12	33	31.0
9	12	67	...	...	12	50	7.8
10	12	42	...	...	22	55	0.3
11	12	0	...	...	30	10	0.1
12	12	0	...	...	34	3	0.2

[a] Test was terminated prematurely because fruit were destroyed by a freeze in January 1985.
[b] Scrapings were deposited on dodine-amended potato-dextrose agar and the values represent the mean number of colonies per 90-mm-diameter petri dish. Four dishes used for each fruit.
[c] Fruit not sampled.
[d] No data. Colonies present but not counted.

to the next crop of fruit by examining young fruit located below and to the side of scab-bearing fruit that had been left on the tree for up to 2 mo after the following bloom.

RESULTS

All of the five or 10 Temple leaves with scab pustules tested at each sampling time yielded viable propagules of *E. fawcettii*. Thus, it could be assumed that when scrapings from pustules on sweet orange fruit failed to yield colonies of *E. fawcettii* on PDA, it was because the fungus was not viable in the stromata, not because the isolation procedure failed.

Results of three survival tests for *E. fawcettii* in scab pustules on sweet orange rind are shown in Table 1. The periods shown from the time of infection to the time of sampling are approximate, because some of the pustules may have been derived from conidia that formed and were dispersed from the original pustules before the fruit became resistant to further attack. The rind becomes immune to scab about 12 wk after petal fall (5) and the earliest time any fruit were inoculated was 4 wk after petal fall. Therefore, none of the scab pustules would have been more than 2 mo younger than the age indicated in Table 1.

In the 1982–1983 season, pustules began to lose their capacity for inoculum production at about 9 mo after inoculation. After 10 mo, none of the scab pustules sampled on any fruit yielded any viable propagules of *E. fawcettii*. No scab pustules were observed on any new fruit, not even where they were in close proximity to diseased fruit that were left on the tree until after fruit-set.

The fruit inoculated in 1984 were tested only until January 1985, because a severe freeze later in that month destroyed the fruit. However, in this test, the scab pustules had already lost some of their inoculum-producing capability by the sixth month after inoculation.

In the 1986–1987 test, all fruit sampled over the first 7 mo after inoculation contained viable propagules of *E. fawcettii*. Thereafter, the numbers declined but never reached zero, which was in contrast to the first test. However, the number of viable propagules still remaining in the stromata after the ninth month was very small compared with earlier in the 1986–1987 season. Despite the continued presence of a few viable propagules in the scab pustules and their possible potential for conidia production, no pustules appeared on the new crop of fruit, indicating that the life cycle of the pathogen had probably been broken.

DISCUSSION

The concept that *E. fawcettii* survives for a limited time in scab pustules on fruit is not new. Winston (7) concluded from isolation studies and field observations that while this pathogen commonly survives through the winter on leaves, it seldom, if ever, overwinters on fruit that were derived from a previous year's spring or summer bloom. On the assumption that if viable mycelia are present in the stromata the pustules must still retain some inoculum-producing potential, it was concluded that some conidia may still be produced on overwintering pustules on fruit even after the next bloom. However, the chances of pathogen survival on fruit lesions seem slight, because no infection of those young fruit in the vicinity of the previous year's infected fruit was observed in the 1982–1983 or in the 1986–1987 tests.

The successful inoculations conducted in these tests and in earlier tests (5), indicated that the sweet orange-infecting biotype of *E. fawcettii* has sufficient virulence to threaten sweet orange production, whereas the results of the fungal survival studies indicated that scab is a rare disease on sweet orange fruit in Florida because the causal fungus rarely survives in sweet orange groves.

Scab on sweet orange in South America has long been attributed to another pathogen, *E. australis* Bitanc. & Jenk. (1), but more study is needed to establish whether such species separation is valid. The rare occurrence of scab on sweet orange in Florida has previously been attributed to the absence of *E. australis* (4). Comparative pathogenicity tests with the sweet orange-infecting biotype of *E. fawcettii* and with *E. australis* would be needed to determine if the latter is more virulent to sweet orange fruit than the former pathogen. Nevertheless, even if *E. australis* is more virulent than *E. fawcettii* on sweet orange fruit, it may not create a problem on sweet orange cultivars in Florida unless there is leaf infection. In Brazil, however, *E. australis* causes little leaf infection (1,3). Therefore, assuming that it is different from *E. fawcettii*, *E. australis* might present little additional threat to sweet orange unless it is able to survive in scab pustules on fruit rind longer than *E. fawcettii* does. Even then, it should only be a threat to late-maturing cultivars, because in Florida only these generally carry mature fruit until after the next spring bloom.

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